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A Measuring Device for Shank Length of Living Birds

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IN CERTAIN studies on the growth of the axial skeleton of the fowl, it was found desirable to make measurements on shank length of individual birds throughout their course of growth. The technic used in previous work on the subject necessitated the killing of birds when a measure of their bone lengths was desired. In order to avoid this a measuring device was designed, a description of which is here presented. This device is of a simple nature, is of inexpensive construction and can be very easily and rapidly operated. Originally its purpose was to provide measurement of shank length only, but its uses are by no means confined to this structure. Other bones which have been dissected out have been successfully measured by the use of this instrument.

The essential parts of the measuring device include a linear scale, a metal shoe and an oscillating pedal. These parts are assembled in and on a metal box (Figures 1 and 2). This box is 29 cm. long, 19 cm. wide, and 12 cm. high and the cover is a tight-fitting slip-on type. These dimensions allow shank length measurements to be made on all breeds of chickens from the day-old stage to maturity.

A metal shoe (K)[2] which receives the hock joint of the birds is made in various sizes to fit the joint of birds of different ages. The size used for adult birds is 5 cm.

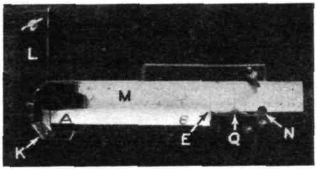

FIG. 1. A measuring device for shank length. The foot is placed against the pedal (E) and pushed forward until the hock joint slips into the shoe (K) and the length is read on the meter scale (M) at the pointer (Q).

high, 3 cm. wide, and 3 cm. deep. It is attached with a wing nut and bolt to an angle iron (L) which in turn is welded to the meter scale (M). The shoe and scale are held in place on the lid of the box by two wing nuts and a lug (N). This construction allows the shoe and scale to slide back and forth at will, permitting approximate adjustment for variations in the length of the structure measured.

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[2] The letters in parentheses refer to the corresponding letters in Figures 1 and 2.

The oscillating pedal (E) is fastened to a smooth square tube (F) by an arm (G). The tube, which is about one-half inch square, rides between the outside races of four standard one-half inch ball bearings

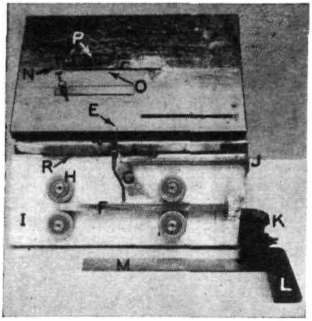

FIG. 2. The instrument partially taken apart to show the inside mechanism which is explained in the text.

(H). The two metal face plates (I)—only one of which is shown—which hold the bearings and the square tubing in place are bolted firmly to the inner races of the bearings. A three-eighths inch extension spring, one end of which is fastened to the arm (G) and the other to the end of the face plate at (J), pulls the pedal toward the shoe exerting a pressure on the shank or other structure, thus enabling a correct measurement to be made.

The assembled mechanism is placed lengthwise in the center of the box and the ends of the face plates are bolted to the short sides of the box. A slit (O) 12 cm. long allows the arm (G) to project through the cover. When the cover plate (P) is removed the cover may be taken off without damage to the pedal (E) and the pointer (Q) which is attached to the pedal by double adjusting nuts permitting a calibration of the instrument. Heavy felt (R) is glued on the edges of both face plates adjacent to the arm (G) so that the edges of the felt come together in front of and behind the arm preventing dust and dirt from entering the oscillating mechanism.

The accuracy of the instrument has been checked by making measurements on living birds and comparing the values obtained to the actual tarsometatarsal length obtained on killing the birds, disjointing and cleaning the shank. Seventy-six Single Comb White Leghorn yearling hens were used for this purpose. The coefficient of correlation between the shank and the tarsometatarsal length was found to be 0.968 ± 0.007, a linear regression relationship obtaining. The standard error of estimate was found to be 0.028, thus indicating a high degree of precision of the instrument.

Shank Length as a Criterion of Inherent Size

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THE writer has elsewhere presented a review of literature on the inheritance of size with particular reference to the domestic fowl (Lerner 1937). Most of the work recorded was based on body weight differences, a notable exception to this being the study made by Maw (1935). The latter used a number of skeletal measurements in crosses of Brahmas with Sebright Bantams and succeeded in demonstrating the existence of sex-linked factor differentials for length of bones in these breeds. Maw as well as Lerner and Asmundson (1932) in a similar study made measurements on prepared bones, dissected out from birds killed for this purpose.

In the course of work on relative growth, the writer found it necessary to take shank measurements of live birds in successive stages of growth. Accordingly, Dr. B. R. Burmester designed a measuring device suitable to make these measurements. This device has been previously described (Burmester and Lerner 1937) and found to be extremely accurate in measuring shank length, since the correlation between the live measurement and the actual length of the tarso-metatarsus found from prepared bones was 0.968 ± 0.007.

Hence, it was possible to use this measuring device to determine the distribution of shank lengths in the University of California flock of Single Comb White Leghorns. This distribution revealed that there are definite differences in size among strains used in recent years in this flock and also emphasized the validity of live shank length as a criterion of inherent body size. Such information as has been obtained on genetic differences for adult size within a breed is presented and the use of the proposed measuring device to facilitate and make more practicable studies of such kind is illustrated.

DATA AND DISCUSSION

A total of 457 Single Comb White Leghorn hens were measured, of which 257 are designated as belonging to the C line. This line comprises the California Station flock, in the ancestry of which no birds bred by Mr. Kimber appear. Stock from several other California breeders has been used in this line, but the generations measured were largely interbred, so that no clear-cut segregation of strains was possible in this line. Of the remainder, 43 birds were first generation crosses between the C line and the Kimber stock (designated as K line) and a further 53 birds were of the backcross to the C line generation. An additional 12 birds comprising a second backcross generation and a first backcross × F_1 cross completed the measurements taken on the station flock. The remaining 92 birds were of straight K line ancestry and, with the exception of three individuals, were measured at the Kimber Poultry Breeding Farm.

The distribution of shank lengths in the

* A sincere appreciation is acknowledged to Mr. John Kimber of the Kimber Poultry Breeding Farm who has kindly coöperated in the study, permitting the taking of measurements on some of his birds.

various groups is presented in Figure 1. It may be observed that neither the C line nor the K line which form the parental groups in this study show a normal distribution.

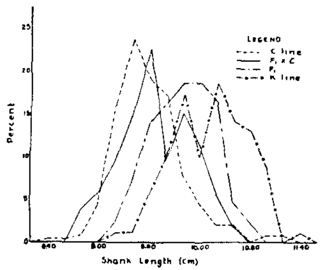

FIG. 1. Frequency of distribution of shank lengths.

The C line, even when the nine individuals at the upper limit are eliminated from consideration, gives a chi-squared value considerably below the .01 point for P, while the K line distribution is distinctly bi-modal. Both of these lines thus fail to give normal frequency distributions. Since neither of them have been selected primarily for size, the distributions presented can only be considered to indicate lack of homogeneity for shank length in the stock. It must be noted, however, that the 92 K line birds formed a random sample originating from 63 different matings, while the K line birds used in the crosses with the C line originated from only four matings.

The F_1 distribution is intermediate between the two parents and deviates from a normal curve in being leptokurtic. The backcross to the C line presents a bi-modal distribution indicating segregation of factors derived from the two lines.

Mean shank measurements for each one of the above groups as well as for the 12 birds in the two other groups represented are shown in Table 1, which also lists the coefficients of variability and the standard errors of these constants. A comparison of the means of the different groups, and a perusal of the differences between them presented in Table 2, shows that the C line and the K line differ genetically for shank length. The hybrids are intermediate, the mode approaching the longer-boned parents. The fact that the backcross generation is closer to the shorter parent than to the F_1 would argue that the increase in the modal F_1 shank length over the midpoint between the two parents is a manifestation of heterosis rather than evidence for dominance of some of the factors involved.

So far as the possibility of sex-linkage is concerned, the reciprocal F_1 crosses on separation showed a difference of 0.13 ± 0.11 cm. in shank length thus indicating lack of evidence for sex-linked factors. Furthermore, the $F_1 \times C$ group consisted of two kinds of backcrosses, in one of which the K line bird was the paternal grandfather and in the other the maternal grandfather. On a

TABLE 1.—*Statistical constants for the different groups*

	Shank length in cm.			Body weight in gms.		
Mating	No. of birds	Mean ± S.E.	Coeff. Var. ± S.E.	No. of birds	Mean ± S.E.	Coeff. Var. ± S.E.
C	257	9.59±0.03	4.28±0.19	201	1666.9± 13.2	11.81±0.60
(F_1 × C) × C	8	9.75±0.21	5.64±1.51	8	1812.0± 88.5	12.92±3.51
F_1×C	53	9.68±0.06	4.24±0.42	52	1713.1± 34.6	14.41±1.46
(F_1 × C) × F_1	4	10.07±0.25	4.27±1.74	4	1659.5±119.1	12.43±5.16
F_1	43	10.00±0.06	3.50±0.38	44	1803.3± 29.1	10.57±1.15
K	92	10.28±0.04	4.14±0.31	—	—	—

TABLE 2.—*Differences between various groups*

Matings compared	Shank length in cm.	Diff./S.E.	Body weight in gms.	Diff./S. E.
K−C	0.69±0.05	13.8	—	—
$K-F_1\times C$	0.60±0.07	8.6	—	—
$K-F_1$	0.28±0.07	4.0	—	—
F_1-C	0.41±0.07	5.9	135.4±31.9	4.3
$F_1-F_1\times C$	0.32±0.08	4.0	90.2±45.2	2.0
$F_1\times C-C$	0.09±0.07	1.3	46.2±37.0	1.2

sex-linked basis the first kind should present a segregation into K line and C line types while the second should yield birds of only the C type. In reality the progeny of the two kinds of backcrosses did not differ in their mean shank length.

Tables 1 and 2 also present information relative to the body weights of the different groups involved, with the exception of K line birds, comparable body weights for which were, unfortunately, not available. This was due to the fact that the station flock was weighed at the average age of 8½ months, while the K line birds were weighed at the time of laying of the first egg. However, it can be clearly seen that body weight is not so satisfactory a criterion of size as shank length, its coefficient of variability being much greater. This is in accord with the earlier findings of Schneider and Dunn (1924), Lerner and Asmundson (1932), and Maw (1936). The differences between the two groups are not as significant on basis of body weight as on basis of shank length. The correlation coefficient between body weight and shank length on 311 birds for which both measurements were available was found to be 0.659 ± 0.032 and strictly linear.

CONCLUSIONS AND SUMMARY

On the basis of shank length measurements of 457 White Leghorn hens it is suggested that:

1. Genetic differences in size can be demonstrated to exist within a breed of poultry.

2. Shank length measured on live birds forms a valid criterion in studies on inherited size differences in fowls.

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Individual Hen and Breed Difference in Egg Weight Losses During Incubation

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IT IS well known that the characteristics of the shell affect the incubation and storage quality of the egg. Any improvement in egg shell quality is, therefore, an economic gain to the poultry industry. Although several investigations have been made to determine the effect of nutrition and other environmental factors on the shell, very little has been done to really understand the shell characteristics of eggs laid by the individual hen or whether or not egg shell quality can be improved by breeding.

Almquist and Holst (1931) made a study of egg shell porosity by allowing an alcoholic dye solution to penetrate into the egg. Their results showed a definite relationship between porosity and weight loss during storage. They reported that egg shell porosity appeared to be nearly uniform for the eggs of a particular hen, but showed differences for different individuals.

Axelsson (1932) reported the results of loss in weight of eggs stored for 24 hours at an average temperature of 22°C. and with 60 percent relative humidity. This investigation involved the eggs from White Leghorns, Rhode Island Reds, Barnevelders, and crosses from the three breeds. He concluded that the physiological characters determining the loss of weight were different in the three breeds and that those differences were hereditary. He regarded the smaller loss of weight as dominant to the larger loss of weight, dependent upon at least one sex-linked and one autosomal factor. However, he stated that the investigation was not complete as it had not been extended to the F_2 generation.

The percentage loss in weight of eggs incubated for 14 days was studied at the National Agricultural Research Center. The eggs were obtained from White Leghorn and Rhode Island Red pullets during the period from April 21 to July 3, 1936. These pullets were fed an all-mash diet that included 1 percent cod liver oil and 2 percent calcite and were permitted access to oyster shell and a limited grass range.

One day after the eggs were laid, they were weighed individually and the weights recorded to the nearest tenth of a gram. The eggs were then incubated in a forced-draft incubator at 99½°F. and 60 percent relative humidity for 14 days, then reweighed. Then they were candled to determine which were infertile and which contained either live or dead embryos. All eggs excepting those that contained live embryos were broken and observed in order to verify the candling records. In the final analysis, only the birds that had either five or more infertile eggs or five or more eggs that contained live embryos or both were included.

A comparison of the mean percent loss in weight for the two breeds is given in Table 1. In each class the eggs from the Rhode Island Reds have a larger mean weight loss than those from the White Leghorns. By referring to tables of values of F and t by Snedecor (1934) it is apparent that the difference in each case is highly significant.

It will be noted the mean loss in weight of the White Leghorn eggs is somewhat

Table 1.—*Mean percent egg weight loss per bird for White Leghorn and Rhode Island Reds*

Breed	Eggs with live embryos		Infertile eggs	
	No. birds involved	Mean percent loss per bird	No. birds involved	Mean percent loss per bird
White Leghorns	127	8.82	38	8.42
Rhode Island Reds	93	9.40	154	9.52
Difference		0.58		1.10
"t"		7.24		9.48

larger in the live-embryo class than in the infertile class. However, when a paired comparison was made for the 29 birds that produced both classes of eggs, the mean loss is slightly larger for the infertile class, but the mean percent difference is only 0.14 which was found to be insignificant.

Although the Rhode Island Red eggs in the two classes have only slightly different means, a paired comparison was also made for the 11 birds that produced both classes. A mean percent difference of 0.084 was obtained which also was found to be insignificant.

An analysis of variance of egg weight loss during incubation is given in Table 2. In each breed for each class of eggs, the variability "between eggs from the same bird" is materially less than the variability "between birds." Again referring to tables of values of F and t by Snedecor (1934), it is obvious that all values obtained for F are highly significant. This means that eggs from the same bird are significantly less variable in weight loss during incubation than eggs from different birds.

As Almquist and Holst (1931) have shown a close relationship between porosity and weight loss during storage and as the present data has shown that eggs from the same bird are less variable in weight loss during incubation than eggs from different birds, eggs from the same bird must also vary less in shell porosity than eggs from different birds. Then egg shell quality as characterized by porosity or weight loss is dependent to a large degree on the individual hen. Work is to be continued in an effort to determine whether the individual hen can transmit this characteristic to her offspring. If this character is found to be inherited, definite breeding plans can be made to improve it.

It is probably true that eggs which lose less weight during incubation will also lose less during storage or when in transit. Loss of weight is mainly a loss of moisture which causes a larger air cell and may be con-

Table 2.—*Variance of egg weight loss during incubation*

Source of variation	Eggs with live embryos						Infertile eggs					
	White Leghorns			Rhode Island Reds			White Leghorns			Rhode Island Reds		
	Degrees of freedom	Sum of squares	Mean square	Degrees of freedom	Sum of squares	Mean square	Degrees of freedom	Sum of squares	Mean square	Degrees of freedom	Sum of squares	Mean square
Between birds	126	4875	38.7	92	1379	15.0	37	716	19.4	153	3228	21.1
Between eggs from same bird	2437	7749	3.2	600	2075	3.5	342	1727	5.0	1080	5275	4.9
Total	2563	12624	4.9	692	3454	5.0	379	2443	6.4	1233	8503	6.9
F			12.1			4.3			3.9			4.3

sidered an indication of the interior quality of the egg. Therefore, any program involving the improvement of weight loss in eggs will be beneficial to the poultry industry and to the consumers of eggs.

CONCLUSIONS

The results obtained seem to warrant the following conclusions:

During incubation eggs from Rhode Island Reds lost a significantly greater amount of weight than eggs from White Leghorns.

Infertile eggs did not differ significantly from fertile eggs in weight loss.

Eggs from the same bird were significantly less variable in weight loss than eggs from different birds.

Weight loss of an egg is a characteristic dependent to a great degree on the individuality of the hen.

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Further Studies of the Effects of Selenium Poisoning on Hatchability*

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MONSTERS in chicks due to the consumption of toxic grains (grains containing selenium) by hens were first reported by Franke and Tully (1935). Since that time three more articles by Tully and Franke (1935), Franke and Tully (1936) and Franke et al (1936) on this same study have been published from this station. These publications have reported the results of experiments which demonstrated that: (a) high chick embryonic mortality in certain localities was due to deformities in the chick embryos; (b) these deformities were caused by the feeding of grains containing selenium to the hens; (c) similar deformities could be produced by injecting inorganic salts of selenium into the air cell of the hens' eggs before incubation; (d) when normal baby chicks were fed a ration containing 65 percent of toxic grains their growth was inhibited, and the egg production of the pullets was both delayed and decreased.

The experiment reported herein was planned with the purpose of answering the following questions:

1. What is the effect of the toxic grains on the weights of the laying hens?
2. What is the effect of the toxic grains on (a) the number, (b) the size or weight, (c) the fertility, and (d) the hatchability of the eggs produced?
3. What is the effect of the toxic grains on the feed consumption of the laying hens?
4. How long after hens are changed from a normal ration to a ration containing 65 percent of toxic grains (15 p.p.m. of selenium, does it take for the toxicity to affect hatchability?
5. How long does it take the toxic effects to disappear after the hens are changed from toxic back to the normal ration?
6. Will the feeding of elemental sulphur at a level of 1 percent in the ration either prevent or alleviate the toxic effects of selenium, or in any way affect the action of selenium?

EXPERIMENTAL PROCEDURE

Three pens of individually mated White Leghorn pullets were fed the control ration for a period of six weeks, and their normal egg production, fertility, hatchability, and so forth were determined. At the end of six weeks pen 2 was changed to the toxic ration and pen 3 was changed to the toxic ration to which had been added 1 percent of powdered sulphur.

Pens 2 and 3 were fed the toxic rations for a period of five weeks and were then changed back to the normal ration for a period of six weeks, at which time the experiment was concluded.

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* The time periods between the initial feeding of grains containing selenium and the appearance of toxic effects in the embryos, and time required *after the termination of "alkali" grain feeding for* the disappearance of toxic effects in the embryos, with a note on the failure of sulphur to inhibit the toxic action of selenium. Published from the Departments of Poultry Husbandry and Experiment Station Chemistry, South Dakota State College, with the permission of the Director of the South Dakota Experiment Station.

† Deceased.

Percentage composition of the all-mash rations

	Control Pen 1	Toxic Pen 2	Toxic plus Sulphur Pen 3
Ground Corn (Toxic, #570, 29 p.p.m. Se)		25.0	25.0
Ground Corn (Normal)	25.0		
Ground Barley (Toxic, #587, 15.4 p.p.m. Se)		25.0	25.0
Ground Barley (Normal)	25.0		
Ground Wheat (Toxic, #607, 30 p.p.m. Se)		15.0	15.0
Ground Wheat (Normal)	15.0		
Wheat Bran	7.5	7.5	7.5
Wheat Middlings	7.5	7.5	7.5
Meat and Bone Meal	8.0	8.0	8.0
Alfalfa Leaf Meal	5.0	5.0	5.0
Buttermilk Powder	5.0	5.0	5.0
Salt	1.0	1.0	1.0
Cod Liver Oil	1.0	1.0	1.0
Total	100.0	100.0	100.0
Selenium Content, pens 2 and 3		(15.15 p.p.m.)	

NOTE: One percent of sulphur added to ration of pen 3.

Individual records on each egg included hen no., date of laying and egg weight. Incubation records were kept as to each hen's hatchability, fertility, embryo mortality, malpositions and deformities.

DISCUSSION OF RESULTS

Control (selenium-free) rations were fed during the first six weeks of the experiment. During this period the hatchability of fertile eggs averaged over 90 percent and remained high for the first few days after changing to the rations containing toxic grains. At six weeks grains containing selenium replaced the normal grains in the rations. Charts 2 and 3 show the rate at which toxic effects appeared in the embryos. On the sixth day after changing to toxic grains embryo

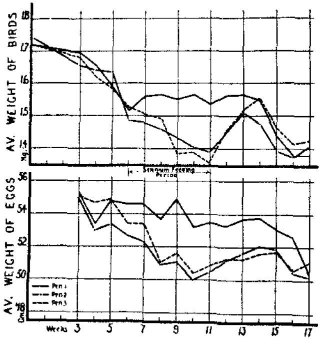

CHART 1

Chart showing average weights of hens and average weights of eggs laid for all three pens throughout the experiment.

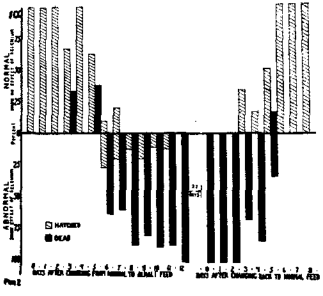

CHART 2

Chart showing incidence of mortality, and deformities of embryos from the eggs from pen 2 when the birds were given "alkalied" grains and the rate of recovery upon changing back to normal ration after 35 days of feeding "alkali" ration.

malformations characteristic of selenium poisoning first appeared, and by the twelfth day none of the eggs from either pen hatched. This condition remained unchanged until normal grains were restored in the rations at 11 weeks. It will be noted that the decrease in hatchability was less abrupt in pen 2 which received no sulphur in the ration.

It is apparent from the results given in chart 1 that the feeding of grains containing selenium caused a loss of weight in laying hens. Chart 1 and Table 3 show that the feeding of grains containing selenium resulted in lowered feed consumption and loss in body weight. There was also a decrease in egg weights which closely corresponded with the decrease in body weight. Upon restoration of selenium-free grains in the ration the feed consumption, body weight, and egg weight increased. Three weeks later, however, all three pens began losing weight. This was probably due to the extremely hot weather.[1]

[1] Beginning June 27 the temperature ranged from about 80° to 110° F. for several days.

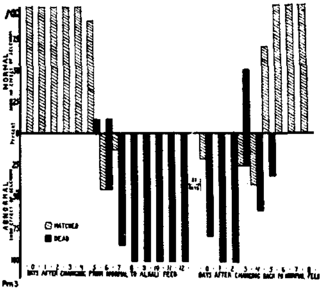

CHART 3

Chart showing incidence of mortality, and deformities of embryos from the eggs from pen 3 when the birds were given "alkalied" grains and the rate of recovery upon changing back to normal ration after 35 days of feeding "alkali" ration.

As indicated in Table 2 the egg production was not appreciably affected by the feeding of grains containing selenium, although it is probable that the continued feeding of these toxic grains would result in decreased egg production, since feed con-

TABLE 1.—*Average weekly egg production per bird*

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pen 1	5.2	5.2	5.2	4.2	4.6	4.6	4.5	4.5	4.4	4.0	3.6	3.0	3.3	3.6	3.3	2.1	2.3
Pen 2	5.0	5.0	5.0	5.2	5.3	5.5	5.1	4.9	3.3	4.1	3.4	4.1	4.3	4.3	4.5	3.6	3.2
Pen 3	4.9	4.9	4.9	4.7	4.0	4.4	4.6	4.5	4.2	3.3	3.1	3.1	3.5	3.8	3.9	3.4	3.0

TABLE 2.—*Average percentage infertile eggs*

Weeks	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
Pen 1	2.0	0.0	0.0	0.0	0.0	0.0	5.9	7.4	6.4	2.3	7.4	10.0	17.9	15.0	—
Pen 2	37.0	10.6	5.0	15.4	13.1	13.6	11.6	7.7	20.0	20.0	32.0	13.8	25.0	31.4	22.0
Pen 3	4.1	4.2	13.3	25.0	5.0	4.5	0.0	0.0	11.0	14.0	5.8	13.1	18.9	12.0	10.3

TABLE 3.—*Average feed consumption, in grams*

Weeks	3*	4	5	6	7	8	9	10	11	12	13	14	15	16	17
Pen 1	148	101	115	102	109	113	102	104	113	93	92	90	69	62	59
Pen 2	205	136	125	101	87	86	90	85	96	103	100	100	86	60	97
Pen 3	209	88	117	106	95	92	84	55	103	118	114	112	94	84	88

* Feed consumption first three weeks.

TABLE 4.—*Types of embryo deformities*

No. days of feeding alkali ration	Pen No.	Percentage of fertile eggs incubated															
						Edema		Twisted		Missing Toes	Upper Beak length				Pro-truding eyes	Eyes missing	
		Hatched	Dead	Alive not hatched	Malpo-sition	Neck	Head	Neck	Legs		Bud	¼	½	¾		One	Both
1–5	2	84.5	11.9	3.6	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
1–5	3	80.0	12.7	7.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
6–12	2	18.6	64.4	17.0	6.8	59.3	16.9	6.8	18.6	0.0	8.5	0.0	10.2	3.4	8.5	0.0	0.0
6–12	3	7.4	77.8	14.8	12.9	46.3	44.4	11.1	25.9	0.0	22.2	0.0	11.1	3.7	11.1	0.0	1.9
13–19	2	2.1	95.7	2.1	17.0	76.6	25.5	19.1	29.8	4.3	0.0	2.1	17.0	6.4	19.1	0.0	4.3
13–19	3	0.0	93.3	6.7	2.2	44.4	17.8	22.2	57.8	4.5	64.4	0.0	0.0	4.5	44.5	2.2	15.6
20–26	2	0.0	93.0	7.0	16.3	62.8	34.9	4.6	34.9	0.0	11.6	11.6	18.6	2.3	23.3	2.3	10.7
20–26	3	0.0	100.0	0.0	8.3	27.8	19.4	2.8	38.9	2.8	55.6	2.8	11.1	0.0	50.0	11.1	13.9
27–33	2	0.0	75.0	25.0	7.1	17.9	25.0	0.0	46.4	7.1	53.6	17.9	3.6	0.0	32.1	0.0	35.7
27–33	3	0.0	93.5	6.5	6.5	19.4	9.7	3.2	29.0	22.6	74.1	0.0	0.0	6.5	35.4	9.7	32.3
34–40*	2	27.5	60.0	12.5	2.5	3.5	22.5	0.0	12.5	7.5	17.5	10.0	7.5	2.5	10.0	10.0	12.5
34–40	3	18.8	75.0	6.2	3.1	31.3	28.1	3.1	31.3	6.3	50.0	0.0	3.1	0.0	25.0	6.3	21.9
41–47	2	91.1	8.9	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
41–47	3	93.3	6.7	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

* Normal ration restored on 35th day.

sumption and body weight were so markedly lowered during this five-week feeding period.

Table 2 shows that fertility was not appreciably influenced by the feeding of grains containing selenium for this short period.

Table 4 gives the incidence of the various types of abnormalities which appeared as a result of feeding the toxic grains. Plate 1 shows typical types of malformations recorded in Table 4. Plate 2 shows the typical wire-haired condition which characterized the few chicks which hatched from eggs laid the second week after changing to toxic grains.

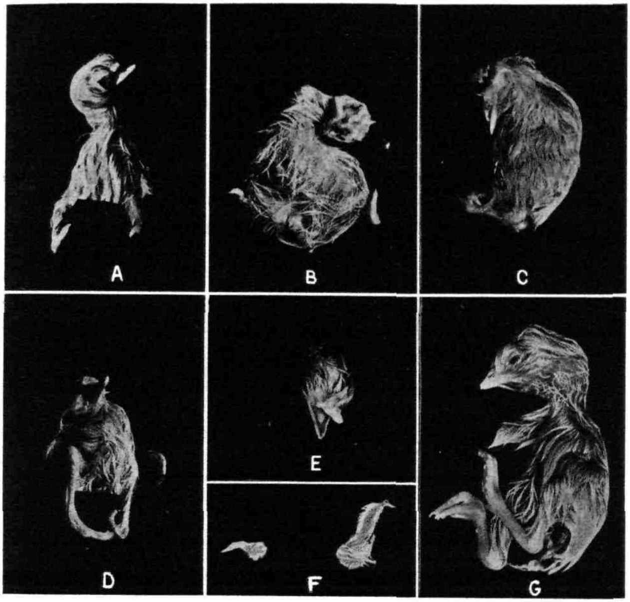

PLATE 1

All show the wirey down

A. Abnormal foot development, only one toe on each leg.
B. Eyes and upper beak missing. Foot development similar to A and D.
C. Hydrocephalia, eyes missing, feet twisted.
D. Upper beak missing, abnormal eye development, foot development similar to A and B.
E. Beak short and twisted.
F. Wings of chick from pen 3 showing difference in rate of development.
G. Chick with edemic neck.

After five weeks, toxic grains were replaced by selenium-free grains. Charts 2 and 3 show the rapid restoration of hatchability in pens 2 and 3. It is apparent that the addition of 1 percent of sulphur to the ration fed to pen 3 did not alleviate the toxic effects of selenium.

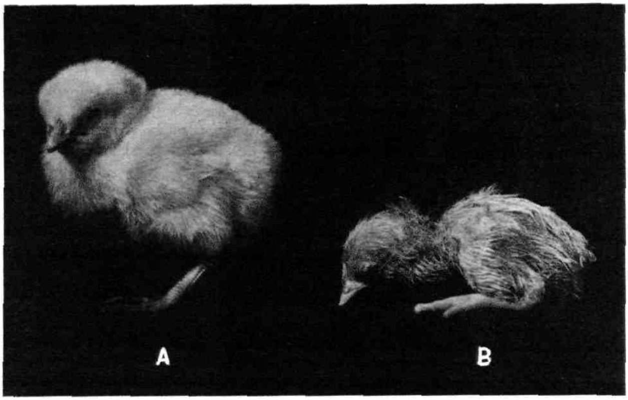

PLATE 2

A. Chick from control pen.

B. Chick hatched second week after changing to "alkali" ration. Notice the "wirey" down and weak condition.

A chemical analysis of a few of the embryo monsters taken from the eggs which failed to hatch revealed a variation of from 6 to 18 p.p.m. of selenium.

Subsequent experiments are being conducted to determine the selenium tolerance of growing chicks and laying hens.

SUMMARY

A balanced ration composed of grains grown naturally but containing 15.15 parts per million of selenium gave the following results when fed for a period of five weeks:

1. Laying hens lost weight during the period over which toxic rations were fed, egg size decreased correspondingly.

2. Egg production and fertility were not appreciably affected during this period.

3. Feed consumption decreased appreciably.

4. Hatchability decreased to zero when toxic grains were fed. No normal chicks hatched after the seventh day of feeding grains containing selenium. On the sixth day after restoring grains containing no selenium, the apparent toxic effects of selenium on embryo development and hatchability had disappeared.

5. Monstrosities characteristic of selenium poisoning clearly show this to be the cause of embryo mortality. No monsters were produced in the control pen.

6. Elemental sulphur used at a level of 1 percent failed to affect the action of selenium.

7. In pen 1 (control) the percent hatchability ranged from 74 to 98 with an average of 87 percent for the entire experiment.

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Paratyphoid in Turkeys*

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An infection in turkeys caused by a *Salmonella aertrycke* type organism has been found responsible for high mortality in poults in several turkey flocks in Idaho. This organism has been isolated from outbreaks in four widely separated localities in the state. In three instances the disease showed up in poults before they were a week old and resulted in the loss of more than 80 percent during the first 10 days. In one outbreak more than 11,000 of 12,000 poults hatched on one farm died of this infection. In a second flock, less than 200 poults were alive out of 2,000 in one brood when they were seven days old. In the third outbreak, only 80 were alive out of 1,500 at 10 days of age.

Rettger, Plastridge, and Cameron (8) in 1933 reported a similar outbreak in turkeys in Connecticut. They found that an organism of the *Salmonella aertrycke* type was responsible for the disease. An organism that appears to be identical with the one isolated by these workers was responsible for the outbreak in Idaho. Lee, Holm, and Murray (6) also isolated this organism from turkeys affected with paratyphoid. These workers reported paratyphoid infection in turkeys to be rather prevalent in Iowa. They were able to isolate the *Salmonella aertrycke* type organism from the ovaries of hens that had been artificially infected with this organism. They also showed that there was a high degree of cross agglutination between *Salmonella aertrycke, Salmonella pullorum,* and *Salmonella gallinarum.*

Salmonella aertrycke, or a very closely related variant, has been found to be associated with various diseases of other birds and animals. For example, Jungherr and Wilcox (4), Khalifa (5), and others have reported a strain of this organism to be the causative agent of paratyphoid in pigeons. Emmel (2) gives an extensive review of the literature on this type of infection in birds and shows that it is rather common. He also shows that a high percentage of birds affected with intestinal parasites have *Salmonella aertrycke* in the intestinal flora. Other birds that have been infected with this organism include canaries and parrots. Newsom and Cross (7) reported an outbreak of paratyphoid dysentery in lambs caused by an organism of the paratyphoid B group, *aertrycke* type. This organism was isolated from both the spleen and intestines of affected animals and was capable of reproducing the disease in normal lambs. More than 30,000 lambs were involved in the outbreak with a mortaltiy of approximately 2,000.

Salmonella aertrycke has frequently been associated with food poisoning in man. Jordan (3) and Savage (9) have found this organism to be the most common cause of food poisoning of this type in the United States and in England. Thus, there may be a rather close relationship between the prevalence of paratyphoid infection in birds and animals and outbreaks of food poisoning in man. Indeed, Damon (1) says that food poisoning of the paratyphoid type in some cases at least, ". . . is due to the use of meat or meat products derived from an animal itself infected with the specific or-

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ganism, because it is a well established fact that *Bacillus enteritidis* and its congeners is pathogenic for some of our food animals as well as for man."

A study of paratyphoid infection in turkeys was made in an outbreak which occured in every brood in one flock throughout the hatching season of 1935. During this season less than 700 of more than 12,000 poults hatched in 12 broods were marketed. In the later broods 80 percent were dead at two weeks of age. Rettger et al (8) report from 10 to 50 percent mortality. Lee et al (6) report losses up to 90 percent during the first five weeks.

The high mortality in this particular outbreak may have been due in part to the low vitality of the poults. This was apparent in that about 20 percent of the breeding stock was suffering from nutritional roup by the middle of the hatching season. Table 1 shows that 251 weak poults were culled from 773 poults in the ninth brood. In addition to low vitality, the poults were overheated, particularly during the latter part of the season. On several occasions the temperature under the hover was checked and found to be over 100°F.

SYMPTOMS AND LESIONS

The disease made its appearance in the first brood from a flock of 500 breeding hens that had been assembled from numerous sources the previous fall. The poults were hatched in a new turkey incubator. This was the first year that a hatchery had been operated on this farm although turkeys had been brooded with reasonable success for several years.

The poults started dying between the first and third day after being taken from the incubator. Table 1 shows the death rate in a typical brood through the eleventh day with a total mortality of 81 percent.

The mortality rate dropped to a few poults daily after the tenth day but continued to some extent until the birds were moved out of the brooder house to clean range.

TABLE 1.—*Typical daily mortality in a brood of 522 of the best poults from a hatch of 773**

Age in days	1	2	3	4	5	6	7	8	9	10	11	Total
Mortality	10	15	35	78	75	81	35	18	12	8	8	375

* The 251 cull poults not included in the table were all dead at the end of ten days.

Weakness was the most common symptom of affected poults. During the first 10 days poults that appeared normal would become weak, assuming a sitting posture, later flatten out on their sides, and die within one or two hours. A small percentage had diarrhea. As the birds became older they showed evidence of sickness for a longer period, characterized by droopiness and occasional diarrhea.

Practically all dead poults were thin, indicating that they had probably been affected for a longer period than indicated by the symptoms. It was revealed by autopsy made from fresh specimens that most of the poults that died under 10 days of age had pneumonia and about half of them had retained watery or coagulated yolk sacs. All birds examined had pale yellow, mottled livers and most of them had pale kidneys typical of Vitamin A deficiency. A common lesion in these young poults was white cheesy plugs lying loose in ceca of normal size. Occasionally slight inflammation was present in the duodenum. Older poults showed less uniform symptoms and lesions. They were usually weak and thin, had a diarrhea and some had retained yolk sacs and pneumonia. No definite diag-

nostic lesion could be detected in the affected poults.

OLDER POULTS SUSCEPTIBLE

Healthy older poults were found to be susceptible to the disease. In July 500 12-week-old poults were purchased and placed on a range with 300 of the older poults that had survived the disease at the ranch. The ground was damp and the birds were not encouraged to range away from the buildings. After two weeks some of the new turkeys began dying. Cultures from the liver, heart, and spleen of these birds yielded the organism typical of this outbreak.

As soon as the purchased birds began dying, 2 percent of "natural" cod liver oil and 10 percent of alfalfa meal were added to the all-mash feed. The hoppers were moved to the range forcing the birds away from the brooder houses. Within a week's time after these changes were made, no more poults died.

One hundred White Rock chicks were brooded in one section of the hot water type house in which the poults were being brooded. They were separated from the poults by a one foot board partition during the first few days. No evidence of the disease developed in any of these chickens.

TYPE OF INFECTION

When the organism responsible for this outbreak was studied in detail, it was found that the morphological, cultural, and biochemical properties were identical with the *Salmonella aertrycke* type organism isolated by Rettger et al (8). The organism was a Gram-negative, non-sporulating rod, 0.5 by 2 microns. It was motile by means of peritrichous flagella. On both nutrient agar and gelatin, typical *Salmonella* growth was observed. Gelatin was not liquefied. A uniform turbidity with a small amount of gray sediment was noted in broth. Litmus milk was turned slightly acid but finally became alkaline. Acid and gas were produced in broth containing dextrose, maltose, levulose, arabinose, manitol, dulcitol, and xylose. The action of the organism on xylose was somewhat delayed but at the end of 96 hours the production of acid and gas was definite. A small amount of acid and gas was produced in broth containing dextrin. Lactose, sucrose, raffinose, salicin, inulin, glycerol, and inositol were not utilized. Lead acetate agar turned brown along the line of stab. Indol was not formed. Nitrates were reduced to nitrites without the formation of gas. The organism was a facultative aerobe and seemed to grow best at 37°C.

Agglutination and agglutinin-absorption tests further indicated that this organism was identical with the *Salmonella aertrycke* type organism isolated by Rettger et al (8).

SOURCE OF INFECTION

In order to determine if the breeding stock was infected with this organism, blood samples were obtained from 26 adult birds and agglutination tests were made. Antigen for the agglutination tests was prepared by washing the bacterial growth from the surface of 18-hour nutrient agar slant cultures with sterile 0.85 percent physiological salt solution. The antigen was then filtered through glass wool, killed by heat, and diluted with sterile 0.85 percent physiological salt solution until it compared in density with tube No. 1 of the McFarland nephelometer. One half of 1 percent phenol was added for a preservative.

Blood sera from the hens were tested by the tube method. Nine, or more than one third of all the birds tested, were reactors in dilutions of 1:50, 1:100, and 1:200. Only five of the 26 birds tested gave negative reactions in all dilutions. Inasmuch as more than one third of the breeding stock reacted to the agglutination test, it seemed desirable to determine if the disease was transmitted through eggs in a manner similar to pullorum disease.

Infertile turkey eggs from the incubator

were available and these were studied first. Twenty-two of these eggs were opened aseptically and from 2 to 5 c.c. of the contents were drawn out with a sterile pipette and discharged into large fermentation tubes of dextrose broth. The tubes were then incubated at 37°C. Growth occurred in only two tubes and was not typical of paratyphoid organisms. No gas was produced in either of the tubes showing growth.

Of 30 eggs that contained dead-in-the-shell turkey poults, however, three yielded the *Salmonella aertrycke* type organism. These eggs were opened aseptically and with a sterile needle some of the fluid surrounding the embryo was streaked on plates of Endo's agar. The embryos were then removed from the shell and autopsied. Cultures were made from the liver and heart blood. Incubation was at 37°C. for 24 hours. Considerable growth of various types of organisms was observed in 24 out of the 30 eggs examined. Usually, when organisms were present inside the shell membrane they were also found in the liver and heart blood of the embryo. Typical colonies were "fished" in each case and the organisms studied in detail. The type of organism most frequently encountered was the colon bacillus. In only one case was the *Salmonella aertrycke* type organism found in pure culture. In the other two cases it was associated with *Escherichia coli.*

About four months after the hens had stopped laying, five that had reacted to the agglutination test were retested and cultured. These birds continued to react in approximately the same dilution as in the previous trial. At autopsy all of these hens had small misshapen yolk sacs on the ovary. In addition one hen showed a small yolk sac in the abdominal cavity and a second hen had a small cheesy yolk sac in the abdominal cavity with some adhesions of the mesentery. Cultures made from the organs of these birds did not yield paratyphoid-like organisms.

CONTROL MEASURES

Early in the course of the disease careful attention was given to cleanliness and sanitation in the incubator and brooder. The incubator was thoroughly fumigated with formaldehyde. However, the poults were not fumigated while hatching or afterwards. No reduction in mortality resulted from this procedure.

Subsequently a brooder equipped with a hardware cloth floor and electric hovers was used on clean ground. The mortality with this equipment was even higher than in the original brooder house, probably because the poults became chilled at night. All poults that were cultured during these attempts to control the disease were found to be infected with the *Salmonella aertrycke* type organism.

Another attempt was made with the June 11 hatch to control or reduce the mortality. This hatch of 773 poults was divided into six lots. Three lots were brooded in the regular brooder house. Of these one lot received medicated drinking water. The second was not fed until the fifth day and the third was the check lot. The fourth was brooded in town free from contact with the farm. The fifth was confined to the isolated hardware cloth brooder and a sixth was shipped to the University of Idaho and was brooded in a battery brooder.

By the sixth day heavy mortality had been experienced in all lots except the second lot which was not fed until the fifth day, and the lot at the University. At this time all lots except the one at the University were combined in the regular brooder house. Twenty-eight days after hatching, there were approximately 100 of the original 735 poults of the combined lots (1 to 5) still alive, which represented a mortality of 87 percent, and two or three birds were dying daily.

No mortality occurred until the seventh day in lot 6 sent to the University. Between the sixth and the fourteenth day 18 poults

out of 38 died. *Salmonella aertrycke* was isolated from all of these poults. Three additional poults died later of other causes. Seventeen of the original 38 poults were thriving at 10 weeks of age. The results obtained in this trial indicate that the infection was present in the poults when they were taken from the incubator. The relatively low mortality in the lot shipped to the University indicated that with good brooding practices approximately 50 percent of the stronger poults could be saved.

Regardless of corrective brooding practices suggested by this trial, succeeding hatches died more rapidly and more extensively than had been the case in earlier hatches.

OTHER OUTBREAKS

In the second outbreak in which the organism was isolated from young poults, approximately one third of the breeding hens reacted when tested with *Salmonella aertrycke* antigen. Two dozen dead-in-the-shell poults and two dozen infertile eggs from this flock failed to yield the causative organism. Five paratyphoid reactor hens from this flock showed a high percentage of abnormal egg yolks in the ovary but failed to yield the *Salmonella aertrycke* type organism. Neither eggs nor adult stock were available for study from the third affected flock.

In a fourth flock where paratyphoid was suspected, 13 out of 24 hens reacted when tested. Six of these hens were autopsied. Tissue from the liver, ovaries, egg yolks, oviduct, and intestines were transferred to broth and Endo's agar and incubated at 37°C. for 24 hours. The *Salmonella aertrycke* type organism was isolated from the ovaries of two birds. One of these birds also yielded the organism from a retained egg yolk.

No reactors were detected in the Idaho Agricultural Experiment Station flock when tested with the same paratyphoid antigen. It is of interest to note that *Salmonella pullorum* was isolated from the ovaries of one hen in flock no. 2.

DISCUSSION

Rettger et al (8) suggested that paratyphoid of turkeys probably was transmitted in a manner similar to that of pullorum disease in chickens. This assumption was further substantiated by Lee et al (6) in that they were able to isolate the causative organism from the ovaries of artificially infected hens. During the course of this investigation the *Salmonella aertrycke* type organism was isolated from the ovaries of naturally infected hens and from dead-in-the-shell poults as well. This seems to confirm the belief of earlier workers that the disease is transmitted from affected hens through the eggs to the poults in the manner of pullorum disease.

Evidence presented to date indicates that paratyphoid infection in turkeys may be rather common. Careful bacteriological studies on specimens where the mortality is high should be undertaken to determine whether or not paratyphoid infection is responsible for such losses. This is particularly true when it is realized that losses in poults not attributed to blackhead are many times not classified. More specifice information concerning methods of detection of the disease in both poults and breeding stock should be determined. This would make possible more satisfactory methods of prevention and control.

In the outbreaks cited in this study and in those reported by Rettger et al (8) major losses occurred in poults under 10 days of age whereas Lee et al (6) reported major losses up to five weeks of age. Further studies should be conducted to determine the age incident of the disease.

SUMMARY

A *Salmonella aertrycke* type organism was found to be responsible for several out-

breaks of paratyphoid in turkeys in widely separated localities in Idaho. Paratyphoid infection of turkeys may be characterized as an endemic septicemic disease of poults.

The poults died rapidly up to 10 days of age with weakness and occasional diarrhea as the common symptoms. No outstanding diagnostic symptoms or lesions were present. Older poults were more chronically affected and responded to good hygienic and management practices.

One third to one half of the hens in the breeding flocks that produced affected poults reacted when tested by the agglutination test with antigen made from the *Salmonella aertrycke* type organism. One flock of hens producing paratyphoid-free poults did not show any reactors.

The *Salmonella aertrycke* type organism was isolated from three out of 30 dead-in-the-shell poults cultured from one flock. The organism was not detected in 23 infertile eggs from the same flock.

The causative organism was isolated from the ovaries of two out of six hens examined. These birds were from a flock in which 13 reacted out of 24 tested.

Attempts to eliminate the disease by fumigating the eggs in the incubator, by isolating the poults in sanitary quarters and by the use of medication in the drinking water were not effective.

Low vitality in the poults at hatching time and overheating them in the brooder house appeared to reduce their resistance to the infection and to raise the mortality rate. One lot of 38 poults brooded at the University in a battery brooder under good conditions showed a mortality of less than 50 percent up to 12 weeks of age. Poults from the same hatch, when brooded on the turkey farm, showed a mortality of approximately 90 percent.

Clean 10-week-old poults brought in from outside sources and ranged with poults from the affected flock died extensively from paratyphoid infection.

The data accumulated in this study seem to indicate that the initial infection was present in the ovaries of the breeding hens; that it was transmitted to fertile eggs and directly to the poults that hatched from such eggs; and that the infection spread rapidly in the incubator and brooder to poults that were not infected at hatching time.

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The Effect of Various Calcium and Phosphorus Salts on the Severity of Perosis

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THE anatomical deformity of the leg bones of young chickens, known commonly as "slipped tendons" or "enlarged hocks" and more recently as perosis (Titus, 1932), has been generally recognized as a disorder distinct from rickets. The symptoms of perosis have been described fully elsewhere (Hunter and Funk, 1930; Payne, 1930, and so forth). The literature on this subject has indicated that it is a nutritional problem, that excessive calcium and phosphorus induce it and that certain feedstuffs, such as rice bran, wheat germ, wheat middlings, and oats, possess a preventive factor. This information has been of practical value in reducing the incidence of perosis. On the other hand, the deformity still appears in the field and in the laboratory. This is perhaps due in part to a difference in the susceptibility of different breeds and strains of chicks (Payne, Hughes, and Lienhardt, 1932; Serfontein and Payne, 1934) and to different environmental conditions (Hunter and Funk, 1930; Hunter, Dutcher, and Knandel, 1931). However, the fundamental factors involved in the cause and prevention of perosis have not yet been established. The present study was undertaken therefore in order to obtain more definite information on this problem.

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EXPERIMENTAL PROCEDURE

Barred Plymouth Rock or Rhode Island Red × Barred Rock crossbred chicks were used in this investigation, since it had been observed in the field and substantiated by Payne, Hughes, and Lienhardt (1932) that the heavy breeds are more susceptible to perosis than Leghorns. In the later experiments, the crossbred cockerel chicks were used in order to eliminate differential sex response.

The day-old chicks were confined on wire floors in electrically-heated battery brooders for an experimental period of six weeks. It was observed that the severity of perosis increased very slightly after six weeks of age. Hence, this period was adequate for the purposes of this investigation. Room temperature was maintained at about 85°F. during the first week and then dropped to about 70°F. A 15-hour day was provided by means of morning and evening lights. The chicks were weighed individually each week and a record of feed consumption was made. The average weight of the chicks in each lot was obtained by averaging the average weights of both sexes. Notations of any symptoms of perosis or any otber disorders were made at the time of weighing.

The method used for measuring the severity of the deformity was as follows. Notations were made weekly of the degree of hock enlargement (H), twisted or crooked

tibia and metatarsus (T), and slipped tendon (S) of each leg of each chick. A subnumber was used to denote severity: 1, designating slight, 2, moderate and, 3, severe. The maximum severity possible for each leg would therefore be H_3 T_3 S_3, or a total of 18 per chick. The percentage severity of perosis was determined for each sex in a pen by the formula:

$$\frac{\text{Total H} + \text{total T} + \text{total S}}{\text{Number of chicks} \times 18} \times 100 = \text{Percentage severity}$$

The pen average was obtained by averaging the percentage severity for both sexes. The final notations of any chicks which died during the experiment with perosis were continued in these compilations each succeeding week.

The basal diet used in this investigation was of the following formula:

Ground yellow corn	63.50
Dehydrated alfalfa	5.00
Dried skimmilk	20.00
Menhaden fish meal	10.00
Salt (Iodized)	0.50
Reenforced cod liver oil	0.25
Refined cotton seed oil	0.75
Total	100.00

This contained on the average 20 percent of protein, 0.95 percent of calcium, and 0.80 percent of phosphorus. The reenforced cod liver oil contained 270 U.S.P. units of vitamin D per gram. Two different lots of yellow corn, dehydrated alfalfa and dried skimmilk and three different lots of fish meal were used. Subsequent results demonstrated that this diet was satisfactory for the production of perosis and yet would support an excellent rate of growth when properly supplemented with certain minerals. Supplementation with minerals was made by direct addition whereas that with feedstuffs was made by replacing an equivalent quantity of ground yellow corn.

RESULTS

In the work reported here, the symptoms of perosis generally occurred in the following order:

1. Slight enlargement of the hock, or tibial-metatarsal joint.
2. Twisting or bending of the distal end of the tibia and the proximal end of the metatarsus.
3. Slipping of the gastrocnemius tendon.

Many cases of perosis were noted, however, in which only enlargement of the hock took place. As this became worse, the shafts of the bones often appeared to become shortened and thickened. At this stage the chicks usually became lame. The slipping of the tendon could be predicated in many instances by its looseness, or lack of tension, over the joint. Chicks with severe enlargement of the hock or twisting of the leg bones usually survived but those with both tendons entirely misplaced often died within a week or 10 days, probably through complete inability to walk. The symptoms of perosis were usually bilaterally symmetrical but not always so. There was no marked tendency for the right leg to be affected to a greater degree than the left. Males were often affected more severely than the females. The faster growing birds were more apt to be severely afflicted on perosis-producing diets.

TABLE 1.—*The relation between the severity of perosis and the percentage of the chicks afflicted*

Severity	Cases	
	Range	Ave.
%	%	%
1–5	8–19	12
6–10	25–40	32
11–15	27–89	57
16–20	40–83	65
21–25	43–94	75
26–30	58–100	83
31–35	78–100	94
36–40	81–100	94
41–55	97–100	99

TABLE 2.—*The level of steamed bone meal necessary to produce the maximum amount of perosis Experiment 1*

Lot	Steamed Bone Meal	Limestone	Ca	P	Ave. Wt. at 6 Wks.	Perosis	
						Cases	Severity
	%	%	%	%	gms.	%	%
1	0	0.7	1.20	0.80	458	66	14
2	1.4	0.4	1.50	1.00	395	97	42
3	2.9	0.1	1.80	1.20	372	100	37
4	4.4	0.0	2.16	1.40	369	97	35

The percentage severity of perosis was compared to the percentage of the number of chicks afflicted in each lot for all experiments conducted to date. The latter figure was also weighted for sex influence by averaging the figures for each sex. The data obtained are given in Table 1. Practically all of the chicks in any lot were affected when the severity was between 25 and 30 percent but the maximum severity went as high as 55 percent. The severity did not exceed this level, probably because of variation in individual response to a lack of the preventive factor and because of the presence of a small amount of this factor in the basal diet.

The first part of Experiment 1 was designed to determine how much phosphorus from steamed bone meal was necessary to cause the maximum severity of perosis with the ratio, Ca:P, held at 1.5:1 by the use of limestone. The amounts fed and the results obtained are presented in Table 2. Each lot was started with 30 Barred Rock chicks. These data show that a level of 1.00 percent of phosphorus and 1.50 percent of calcium caused as much perosis as the two higher levels. Growth was reduced as the severity of perosis increased.

In the same experiment a series of three lots was included to determine whether the results of Insko, Sowell, and Lyons (1934), showing that the ratio, Ca:P, is of no particular significance in this disorder, could be substantiated. Previously Sherwood (1932) had concluded that an insufficient amount of lime favored the development of perosis. The results, presented in Table 3, are in general agreement with those of Insko et al. although the highest ratio produced slightly less severe deformities. The significance of the use of limestone will be discussed later.

Another question investigated in the first experiment was the effectiveness of different sources of phosphorus in producing perosis when fed at a phosphorus level of 1.2 percent, with the calcium adjusted at a level of 1.8 percent by the use of limestone. The sources of phosphorus used were steamed bone meal, technical tri-calcium phosphate, c.p. mono-sodium phosphate and technical mono-calcium phosphate. The results are

TABLE 3.—*The effect of increasing the ratio of calcium to phosphorus on the incidence of perosis Experiment 1*

Lot	Steamed Bone Meal	Limestone	Ca	P	Ca:P	Ave. Wt. at 6 Wks.	Perosis	
							Cases	Severity
	%	%	%	%		gms.	%	%
3	2.9	0.1	1.80	1.20	1.5:1	372	100	37
5	3.0	1.8	2.40	1.20	2.0:1	350	100	36
6	3.2	3.6	3.00	1.20	2.5:1	354	100	27

given in Table 4. The c.p. mono-sodium phosphate caused the most severe perosis, closely followed by steamed bone meal and the technical tri-calcium phosphate. On the other hand, the technical mono-calcium phosphate was found to possess a marked preventive action. When the limestone used to adjust the calcium level was omitted, thus dropping the calcium level to 1.20 percent, perosis was entirely prevented by the technical mono-calcium phosphate. This indicated, either that a more acid salt of tri-calcium phosphate in a muffle furnace to remove the adsorbed ammonia. The yields of all three salts ranged from 100 to 103 percent of the theoretically correct yield.

These prepared salts were added to the basal diet on an equivalent phosphorus basis, in one case not equalizing the calcium and in another adjusting to the same calcium level as in the tri-calcium phosphate lot by the use of c.p. calcium carbonate. Since Hunter, Dutcher, and Knandel

TABLE 4.—*The effect of various sources of phosphorus on the severity of perosis*
Experiment 1

Lot	Source	Ca	P	Ave. Wt. at 6 Wks.	Perosis	
					Cases	Severity
		%	%	gms.	%	%
1	Basal diet	1.20	0.80	458	66	14
3	Steamed bone meal	1.80	1.20	372	100	37
7	$Ca_3(PO_4)_2$, tech.	1.80	1.20	414	94	34
8	$NaH_2PO_4 \cdot H_2O$, c.p.	1.80	1.20	374	100	46
9	$CaH_4(PO_4)_2 \cdot H_2O$, tech.	1.80	1.20	483	48	11
10	$CaH_4(PO_4)_2 \cdot H_2O$, tech.	1.20	1.20	480	0	0

calcium and phosphorus was effective in preventing perosis, or that some contaminant was present which possessed a preventive action.

The first possibility was investigated in Experiment 2, using 10 male and 10 female crossbred chicks in each lot. Mono-, di-, and tri-calcium phosphates were formed by combining the proper amounts of c.p. phosphoric acid and of c.p. calcium carbonate. Spectrographic examination showed these materials to be practically pure. In order to get as complete a reaction as possible, the calcium carbonate was calcined, hydrated and boiled with the phosphoric acid in water for four to five hours. In the case of the tri-calcium phosphate, the mixture was treated with an excess of ammonium hydroxide to drive the reaction to completion. The salts were finally dried and weighed. It was necessary to heat the (1931) and Payne, Hughes, and Lienhardt (1932) had found calcium carbonate to be causative, whereas Insko, Sowell, and Lyons (1934) had found it to be neutral, sufficient c.p. calcium carbonate was fed in one lot to make the ratio, Ca:P, 2:1. The effect of an acid and a basic salt of calcium was noted by feeding equivalent amounts of the hydroxide and of the chloride prepared from this same carbonate.

The results are shown in the first part of Table 5. It is evident from these data that the salts of calcium and phosphorus and of calcium were equally effective in producing perosis of about the same severity as that produced by the steamed bone meal. The acidity or basicity of the salts used did not have any influence on the amount of perosis produced except possibly in the case of calcium chloride. This salt was slightly less effective than the other salts but this was

probably due to the detrimental effect which it had on growth.

To test the validity of these results, mono-calcium phosphate was again prepared and fed as in Experiment 2. Each lot contained 16 crossbred cockerel chicks. The calcium carbonate and phosphoric acid were each fed separately, using sufficient to make the combined calcium and phosphorus content the same as in the mono-calcium phosphate lot (2.37 percent).

The data are given in the second part of Table 5. The results substantiate the previous finding that calcium carbonate aggravates the intensity of perosis. On the other hand, phosphoric acid given to lot 4, did not seem to be effective. The results in this lot are of little value, however, since an examination of the calcification showed that the chicks were suffering from high phosphorous-low calcium rickets. An examination of the leg bones in the other lots in this experiment revealed slightly abnormal calcification in a few individuals. The presence of this condition was unexpected as it had not been noted in the initial experiments on perosis. Despite this slight complication, however, good growth and clear-cut results, except on phosphoric acid, were obtained in this experiment.

As a result of these experiments, it seems evident that the preventive action of the technical mono-calcium phosphate used in Experiment 1 was not due to the form of the salt, but to impurities. The results of an investigation of various contaminating elements of which a preliminary report (Wilgus, Norris, and Heuser, 1936) has been made, will be published in full in another paper.

TABLE 5—*The effect of various sources of calcium and phosphorus on the severity of perosis*

Lot	Source	Ca	P	Ave. Wt. at 6 Wks.	Perosis	
					Cases	Severity
		%	%	gms.	%	%
	Experiment 2					
1	Basal diet	0.98	0.75	431	83	19
2	Steamed bone meal	1.78	1.14	374	100	36
3	$CaH_4(PO_4)_2 \cdot H_2O$, c.p.	1.23	1.17	360	95	29
4	$Ca_2H_2(PO_4)_2$, c.p.	1.49	1.17	362	100	34
5	$Ca_3(PO_4)_2$, c.p.	1.75	1.16	364	100	34
6	$CaH_4(PO_4)_2 \cdot H_2O$, c.p. $+CaCO_3$,c.p.	1.74	1.16	361	90	30
7	$Ca_2H_2(PO_4)_2$, c.p. $+CaCO_3$	1.75	1.16	340	100	34
8	$CaCO_3$, c.p.	1.60	0.74	357	95	33
9	$Ca(OH)_2$, c.p.	1.62	0.74	396	95	31
10	$CaCl_2$, c.p.	1.61	0.73	319	80	26
	Experiment 3					
1	Basal diet	0.97	0.78	487	87	21
2	$CaH_4(PO_4)_2 \cdot H_2O$, c.p.	1.21	1.17	442	94	32
3	$CaCO_3$, c.p.	1.59	0.77	500	81	37
4	H_3PO_4, c.p.	0.95	1.42	273	94	21*

* These birds were suffering from rickets.

DISCUSSION

The probability, that some contaminating material in the impure mono-calcium phosphate may be effective in preventing perosis, presents a new method of approaching this problem. That this occurred in an inorganic salt indicates that it is inorganic in nature.

Its relation to the preventive factor in certain cereal products is not clear unless the latter is likewise inorganic.

This investigation confirms the reports of Hunter, et al (1931) and of Payne, et al (1932) that pure calcium carbonate aggravates the occurrence of perosis. The failure of limestone to do so in the first experiment was probably due to the fact that sufficient bone meal was already present to cause perosis of maximum severity. The slight alleviating action of this limestone at the highest level fed and the similar effect of pulverized oyster shell reported by Sherwood (1932) may have been due to active impurities present. The use of too high levels of calcium and phosphorus and the presence of impurities in the mineral supplements used have probably complicated investigations of the effect of the ratio, Ca:P, on the incidence of perosis.

This series of experiments also confirms the aggravating effect of phosphorus on the severity of perosis, in spite of the unsatisfactory results obtained by feeding phosphoric acid. Direct evidence that phosphorus is causative was provided by feeding c.p. mono-sodium phosphate, since experimental evidence and prolonged experience show that sodium is not involved. These results lend weight to Milby's (1933) interesting statistical study of this phase of the problem in which he found that the level of phosphorus in the diet was closely correlated with the incidence of perosis.

SUMMARY

1. The severity of perosis was as great when the calcium and phosphorus content of the basal diet was increased to 1.5 and 1.0 percent respectively by the use of steamed bone meal as when higher levels were fed.

2. C.p. calcium carbonate, hydroxide and chloride as well as c.p. mono-, di-, and tricalcium phosphate increased the severity of perosis to about the same extent as steamed bone meal when added to a basal ration low in the preventive factor. C.p. mono-sodium phosphate was somewhat more effective in aggravating this deformity.

3. The preventive action of a technical grade of mono-calcium phosphate was not due to the form of the salt but to some contaminating material presumably inorganic in nature.

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Relation of Grit to the Development of the Gizzard Lining in Chicks*

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THE occurrence of crater lesions in the linings of the gizzards of young chicks has been studied by various investigators (Holst and Halbrook 1933, Dam 1934, Dam and Schönheyder 1934, Almquist and Stokstad 1935) and has been ascribed to a deficiency in the ration. Studies on the nature of the specific factor involved have been reported by us (1936) and by Almquist and Stokstad (1936). In attempting to extend our investigations on this subject, we encountered a serious obstacle in the development of a second type of abnormality characterized by a very considerable thickening or swelling of the gizzard lining, which not only tended to conceal any crater lesions which might be present, but also led in some cases to the appearance of cracks in the lining which simulated true crater lesions. Such gizzard linings were softer and lighter in color than the normal and showed very marked ridges.

The occurrence of this condition did not seem to be correlated with the occurrence of crater lesions. The problem of preventing the condition was approached from various angles, but with no success until the effect of grit was tried. The results of the first experiments with grit were not decisive but seemed to indicate that feeding grit reduced the incidence of swollen linings. A review of the literature on grit did not bring to light any reference to thickened or swollen gizzard linings; in fact, several investigators expressed the opinion that grit did not serve any important function in the physiology of the chick. Bethke and Kennard (1926) and Buckner, Martin, and Peter (1926) concluded that chicks did not benefit by the addition of grit to the ration, and Carrick, Hauge, and Prange (1926) and Waite (1935) obtained good growth when chicks were fed a gritless ration. On the other hand, Wheeler (1903) and Platt and Stephenson (1935) reported that a ration containing grit gave better growth than the same ration without grit; and Jull (1915) obtained more economical gains when grit was supplied.

It is believed that the results of our experiments are of some importance in that they indicate a specific function of grit, and they point to an improvement in the method of assay for the anti-gizzard-erosion factor.

EXPERIMENTAL

Day-old White Leghorn chicks for these experiments were secured in part from the Department of Poultry Husbandry and in part from commercial hatcheries. The details of procedure were essentially the same as described in our previous paper on the anti-gizzard-erosion factor (Bird, Kline, Elvehjem, Hart, and Halpin, 1936). The

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chicks were killed at five weeks of age and the gizzards graded according to the degree of thickening or swelling of the lining as none or slight, marked, or severe.

The composition of the two basal rations used in these experiments was as follows:

Ration 456		*Ration 360*	
Dextrin, heated 120°C., 24 hrs.	60	Oat groats	80
Casein, heated 120°C., 24 hrs.	18	Casein, heated 120°C., 24 hrs.	15
Peanuts	10	NaCl	1
Salts 1	5	$CaCO_3$	1
Brewer's yeast	2	$Ca_3(PO_4)_2$	1
Baker's yeast	3	Baker's yeast	1
Cod liver oil	2	Cod liver oil	1

Ration 456 is the basal ration used in the study of the anti-gizzard-erosion factor, and chicks receiving this ration without any supplement usually develop both crater lesions and swelling of the lining. The oat groats in ration 360 contains an ample supply of the anti-gizzard-erosion factor, and this ration proved very satisfactory for the study of the *swollen linings* uncomplicated by crater lesions. For the most part the grit supplements were fed as 5 percent of the ration, being substituted for an equal amount of dextrin or oat groats. In a few experiments the grit was fed ad libitum. In all the experiments reported here a medium size granite grit was used. Some of the first grit experiments involved the feeding of a more finely ground granite grit, but this was not well retained by the chicks, and the results were not satisfactory.

RESULTS AND DISCUSSION

The data on growth of the chicks and occurrence of swollen gizzard linings are given in the table. These results show that the grit in most cases prevented and in all cases alleviated this abnormality. Only in the case of ration 456 and the same ration supplemented with 32 percent whole oats did the grit supplement fail to give completely satisfactory protection. The results obtained with ration 456 may be explained on the basis that severe crater lesions occur on this ration, thus making it more difficult to distinguish swollen linings from those that are not. Also it has been observed that gizzards showing severe crater lesions usually contain only a small amount of grit, possibly due to a failure of retention, since the muscular walls of these gizzards are always thin and flabby. The very poor showing of the group receiving ration 456 supplemented with grit and 32 percent whole oats must remain unexplained, but even here the grit seemed to effect some improvement in the gizzard lining. In a number of experiments there was better growth on the grit ration than on the gritless ration, but this was not always the case, as shown by the figures in the table. In those experiments that showed poorer growth on the grit ration, the greatest retardation of growth was during the first week or two and it was observed that there was usually great variation in weight among the chicks of such groups. It was thought that these results might be accounted for by the tendency of the grit to aggravate crater lesions already present in the gizzard linings at the time of hatching. Such lesions have been found to occur in some of the chicks of practically every hatch examined, sometimes in only a few chicks, sometimes in a large proportion of the hatch. This idea led to the experiment in which one group received ration 360 with no grit, another group received grit from the beginning, another after one week, and another after two weeks. The figures show some advantage in growth for the groups receiving grit after one or two weeks. It should be pointed out, however, that these results are not in disagreement with those reported by Bethke and Kennard (1926) who obtained better growth on the grit ration up to the age 14 weeks and, in fact, had greater differences in weight be-

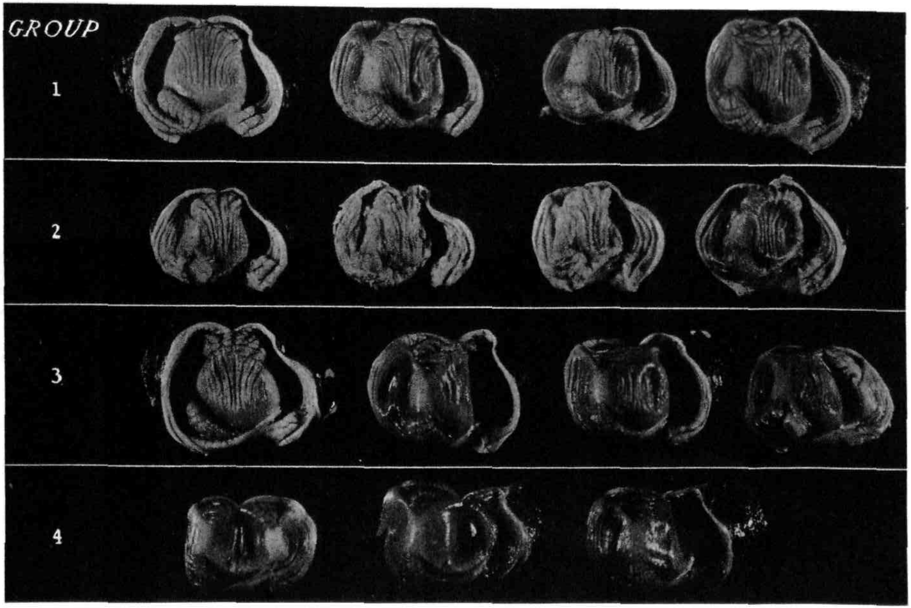

Fig. 1. Gizzards of chicks receiving Ration 360 coarsely and finely ground, with and without grit. Group 1—ration 360 coarsely ground. Group 2—ration 360 finely ground. Group 3—ration 360 coarsely ground +5 percent grit. Group 4—ration 360 finely ground +5 percent grit.

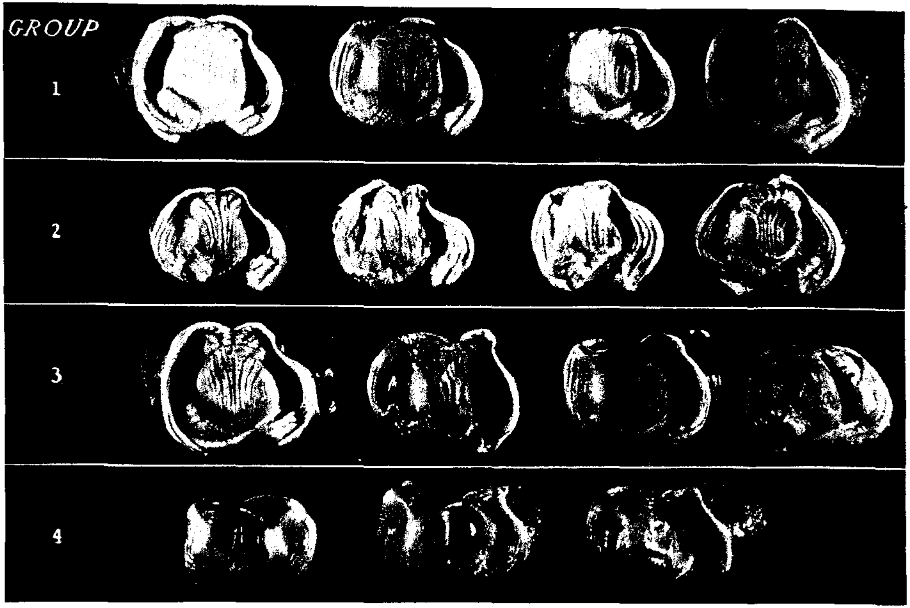

FIG. 1. Gizzards of chicks receiving Ration 360 coarsely and finely ground, with and without grit. Group 1—ration 360 coarsely ground. Group 2—ration 360 finely ground. Group 3—ration 360 coarsely ground +5 percent grit. Group 4—ration 360 finely ground +5 percent grit.

tween chicks on grit rations and those on no grit rations than any reported here. It was the superior growth of the chicks on the gritless ration between the fourteenth and twenty-eighth weeks that led them to the conclusion that there was no benefit from the use of grit.

The fact that swollen gizzard linings did not always occur on the gritless rations led to the experiments on the effect of coarseness of the ration and it was found, as shown in the table, that a coarsely ground ration prevented the thickening of the lining as well as did a ration containing grit. The gizzards from the chicks in the experiment on ration 360 coarsely and finely ground, with and without grit, are shown in Figure 1. There is thickening of the gizzard linings only in group 2, which received the finely ground ration without grit. Waite (1935) expressed the idea that coarse grain particles might perform the function of grit, and these experiments seem to indicate that this is true. From this standpoint, it is of interest that in the experiments of Bethke and Kennard (1926) cracked grains were supplied after the tenth week; and Buckner, Martin, and Peter (1926) who found no advantage from feeding grit, fed a coarsely ground mash and a grain mixture containing cracked corn.

The importance of the experiments described here from the standpoint of future work on the anti-gizzard-erosion factor is obvious. The addition of 5 percent grit to the experimental rations when the chicks are two weeks of age makes for more marked differences between gizzards showing crater lesions and those showing none, since the lesions are accentuated by the addition of grit. Since the grit is not entirely effective when severe crater lesions are present, such gizzards may show thickening of the lining as well as lesions, but gizzards that are free of lesions should also be free of swollen linings.

Table 1.—*Effect of various rations on the swelling of chick gizzard linings*

Basal ration	Supplement	Av. wt. 3 wks.	Av. wt. 5 wks.	Degree of swelling		
				None or slight	Marked	Severe
		gms.	gms.			
360	None	94	176	3	2	4
360	5% grit	103	196	15	1	
456	None	62	138	1	5	3
456	5% grit	86	148	7	2	2
456	32% whole oats	108	207			3
456	32% whole oats+5% grit+grit ad lib.	82	143		2	1
456	10% vac. dried lung	140	270	1		3
456	10% vac. dried lung+5% grit	126	245	3	1	
360	None. Ration finely ground	96	171		1	3
360	None. Ration coarsely ground	112	186	4		
456	None. Ration finely ground	94	145	1	3	
456	None. Ration coarsely ground	99	127	4		
360	None	97	190	1	1	1
360	5% grit	91	193	3		
360	5% grit added after one week	107	202	3		
360	5% grit added after two weeks	120	220	3	1	

SUMMARY

An abnormal thickening or swelling of the gizzard lining, distinct from crater lesion, has been found to occur in young chicks fed a finely ground, gritless ration. The condition may be prevented by feeding grit or a coarsely ground ration.

There is evidence that poor growth is associated with this abnormality and hence that growth may be improved by supplying grit; but growth results may be complicated by the bad effect of grit on chicks having crater lesions.

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Factors Producing and Preventing Perosis in Chickens

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PEROSIS, a term which has been rather generally accepted, refers to a defective leg and bone formation occurring in battery fed chickens. Articles and bibliographies dealing with this subject have appeared so frequently in recent years that no attempt will be made to review the history of this condition. References will be made to articles that have a direct bearing upon the specific problems here discussed.

SOURCE OF DATA

Five years ago, while making a study of the calcium and phosphorus distributions in the blood of young chickens, a few cases of defective hocks were observed. Since it was believed at that time that the condition was closely related to rickets, analyses were conducted to determine whether or not certain fractions of the calcium or phosphorus in the blood differed sufficiently from the normal to explain the cause of the disorder. After making careful external examinations of the legs, X-ray studies of the bones, and hundreds of analyses of the blood, as well as the bones of the chickens, the conclusion was reached that the factors responsible for the cause and cure of rickets were not effective in perosis. Excessive minerals, if of any importance at all, only aggravated the condition. At that time a ration was used composed of ground yellow corn 70 percent, cottonseed meal 5 percent, alfalfa leaf meal 10 percent, dried skimmilk 5 percent, casein 6 percent, $CaCO_3$ 2 percent, NaCl 1 percent, and cod liver oil 1 percent. In using this ration the greatest hindrance to the work was that only a few chicks developed typical defective legs. For this reason, it was never possible to compare large groups where all the individuals in each group were alike. Shortly after that time this ration was changed slightly to make it conform more nearly to that used by certain other investigators. The percentage composition of the new ration was as follows: yellow corn 66.25, wheat gray shorts 5.00, alfalfa leaf meal (dehydrated) 3.00, dried buttermilk 15.00, meat and bone scraps 5.00, bone meal 4.00, cod liver oil 1.00, and sodium chloride .75. The results were gratifying and in certain cases practically every test-chick developed certain defects in the legs. This change in rations made it much easier to study the cause and cure of this condition. The new ration, as given, is referred to in this article as the control or basal feed. From the time the difference in results of two rations so similar in many respects was observed until this time, a period of over five years, many modifications and supplements have been added and thousands of chickens observed. The first of these changes was based upon an attempt to lower the mineral mixture by supplementing the meat meal with other proteins. It was found that by replacing half of the corn with whole ground wheat or rice bran the occurrence of perosis was lowered. An attempt was then made to find some substance in corn, possibly the phospholipids, that might aggravate the condition, or some material in wheat or rice bran that would prevent its occurrence. For this

reason, water, alcohol, and ether extracts were made from yellow corn, whole wheat, wheat bran, wheat embryo, rice bran, and alfalfa and added to the basal ration in the form of a drink. Part of these rations and a summary of results are presented in Table 1. It might be briefly stated that in no case was the condition aggravated by any of the corn extracts and thus it was evidently produced by a deficiency of other components rather than an excessive amount of corn. It was likewise observed, and this fact has been substantiated time after time, that a water extract of rice bran

TABLE 1.—*The effect of various supplements on perosis*

No. chicks	Ration	Supplement	Bent	True perosis	Total defective
			Percent	Percent	Percent
		May, 1935			
100	Basal		37	58	95
100	"	2% Na_2CO_3	24	47	71
100	"	1% Na_2SiO_3	13	75	88
100	"	1% H_3PO_4	0	74	74
100	"	8% Fiber	6	87	93
		November, 1935			
50	Basal		12	64	76
50	"	Alcoholic extract of wheat embryo	40	48	88
50	"	Alcoholic extracted wheat embryo	20	48	68
		December, 1935			
50	Basal		4	79	83
50	"	Water extract of wheat embryo	16	76	92
50	"	Water extract of rice bran	18	0	18
50	"	10% of autoclaved yeast	17	46	63
50	"	10% of yeast	4	79	83
		February, 1936			
35	Basal		4	96	100
35	"	Water extract of wheat embryo	8	92	100
35	"	Water extract of rice bran	17	20	37
35	"	Water extract of alfalfa flour	12	72	84
35	"	Autoclaved yeast	13	71	84
35	"	Ash of wheat embryo	12	72	84
		June, 1936			
40	Basal		8	92	100
40	"	Minus bone meal	9	63	72
40	"	Rice bran tea	8	4	12
40	"	Ash of rice bran	29	68	97
40	"	Cottonseed meal replacing meat scraps	20	72	92
40	"	15% of rice bran	26	44	70
40	"	10% of meat scraps replacing bone meal	23	43	66
40	Old perosis ration				
		December, 1936			
25	Basal		16	60	76
25	"	Water extract of wheat embryo	16	52	68
25	"	Water extract of wheat bran	0	100	100
25	"	Water extract of wheat gray shorts	8	56	64
25	"	Water extract of rice bran	0	12	12
25	"	Ash of 70 pounds of rice bran	0	0	0
25	"	Ash of 70 pounds of wheat gray shorts	20	4	24
25	"	Ash of 70 pounds of wheat bran	8	48	56
25	"	Plus .02% $MnCO_3$	4	0	4
25	"	Plus .02% $MnCl_2$	0	0	0
25	"	Plus 1% of $MnCO_3$	0	0	52% died
25	"	Low protein	16	8	24
25	"	Casein replacing bone meal and meat and bone scraps	0	0	0

would prevent the occurrence of the condition. Some relief was noted with wheat embryo extract; a slight relief from wheat gray shorts extract; but only slight benefit resulted from wheat bran and alfalfa leaf meal extracts. Further tests, in which yeast and autoclaved yeast were used, produced negative results, thus disproving the possibility of this preventive factor being a water soluble vitamin of the B or G complex. From an examination of the accumulated data it becomes evident that the basal ration could be depended upon to produce perosis in from 76 to 100 percent of the observed battery brooded chicks, and that a water soluble, heat stable extract of rice bran, evidently not in the nature of a vitamin, was effective in preventing the occurrence of this condition. The ash of this extract, equivalent to 5 percent of the entire ration, seemed to be ineffective. The effectiveness of this preventive, the exact nature of which was not known, was reported by Heller and Penquite (1936) at the annual meeting of the Poultry Science Association in August, 1936. At the same time Wilgus, Norris, and Heuser (1936) reported the discovery of a preventive in the form of a calcium compound contaminated with manganese, and the theory that manganese and possibly other ions such as iron might be responsible for prevention. At their suggestion our rations have since been examined for their manganese content with the result that there is evidence to support their theory. The results of our observations of 350 chickens are recorded in the latter part of Table 1. It is hoped that the data so reported may be of assistance in aiding other investigators in forming their final conclusions regarding the function of necessary factors in the feed.

The results are easily interpreted. Again the water extract of rice bran was curative, but not as potent as in former trials, possibly due to the source of the sample. The wheat bran extract again failed to protect. The results, as previously reported, are not entirely in accord with the report of Sherwood (1936), possibly due to the source of wheat bran or other factors.

Previously, the ash from a 5 percent equivalent of extract had not effected a cure. In this trial the ash of 70 pounds of wheat bran added to 100 pounds of the basal mixture proved to be a preventive. The ash of wheat embryo was slightly effective, while the ash of an equal quantity of wheat bran was of little value. It must be recognized that normally this amount of ash would never be used, but the quantity was included to give the mineral theory the benefit of the doubt.

DISCUSSION AND SUMMARY

A five-year experiment consisting of careful external examinations of the legs, X-ray studies of the bones, hundreds of analyses of the blood and the bones of chickens, demonstrates that the factors responsible for the cause and cure of rickets did not affect perosis. In the majority of the trials excessive minerals only aggravated the condition.

A ration was developed that was effective in producing perosis in 76 to 100 percent of the chicks used.

A drinking solution consisting of the water extract of rice bran prevented the occurrence of defective legs.

Ether, alcohol, and water extracts of components of the basal ration, when added to the basal ration, did not aggravate the condition, leading to the conclusion that a missing factor must be responsible.

Water extracts of wheat bran, wheat gray shorts, wheat embryo, and alfalfa did not prove very effective.

The chickens consuming water extracts of rice bran had larger, smoother, and better colored legs than the controls. The growth of the chickens was increased, dis-

proving the statement, often made in the past, that the most rapidly growing chickens were most susceptible to abnormalities.

The ash of 70 pounds of rice bran added to 100 pounds of the basal ration prevented the occurrence of perosis.

A correlation seems to exist between the manganese content and the curative properties of the rations, lending evidence to the theory of others that possibly this ion might be the deficient factor.

High percentages of manganese seem to be detrimental to growth. The fact that some of the best growth and protection came from rations having no manganese addition indicates that there may be other factors involved.

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The Nitrogen Balance of Laying Pullets

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RECENT experiments (Prentice, 1933)[1] concerning the protein requirements of laying pullets have demonstrated that under field conditions cereal rations supplemented by 0.5-1.0 percent salt and oyster shell ad libitum gave as good egg production as similar rations containing various levels of protein-rich supplements. The results of these experiments suggest that it is unnecessary under field conditions to include protein-rich supplements in the ration of laying birds and that the protein requirements for maintenance and production are lower than the accepted standards (Halnan, 1934). Though these experiments have very considerable practical importance in that they enable the poultry-farmer to use less expensive rations they have little scientific value in estimating the nitrogen requirements of laying birds, for under the conditions of the experiment any deficiency in the ration might have been made good by the consumption of protein-rich supplements such as grass, worms, and so forth.

Ackerson, Blish, and Mussehl (1923) have measured the average daily endogenous loss of nitrogen in mature non-moulting Rhode Island Red hens by means of balance experiments. Halnan (1925) has reported the results of balance experiments carried out with non-laying and laying pullets over a period of 14 weeks. He found that there was a marked storage of nitrogen immediately prior to egg production and that the birds remained on a positive nitrogen balance during the greater part of the experiment. He concluded that the storage of nitrogen for egg production did not commence until a week or so prior to egg production and that the nitrogen required for egg production can be largely, if not entirely, supplied by the food consumed during the period of egg production. The results of a protein balance experiment carried out by Willcox (1934) on two Rhode Island Red hens over a period of 10 weeks suggest that excellent egg production can be obtained from rations containing less protein than the present accepted standards. During an experimental period of 70 days bird 1 produced 54 eggs and retained 8.77 gms. of nitrogen and bird 2 produced 61 eggs and retained 2.14 gms. of nitrogen. The author concludes that the retention of nitrogen for egg production does not appear to be correlated with the output of egg nitrogen.

In order to gain further information on the protein requirements of laying birds the following balance experiment was carried out on two birds fed on a low-protein diet.

EXPERIMENTAL WORK

Stock. Two Rhode Island Red pullets that were coming into full lay were selected at the commencement of the experiment but one of these birds had to be discarded after 14 weeks owing to unsatisfactory egg production and poor body condition. A post-mortem examination of this bird showed that it was affected with intestinal tumors and it was finally decided to reject the results obtained from it and carry out another trial with a bird that had been kept under identical conditions from the commencement of the experiment.

[1] See also "Progress Report from the National Institute of Poultry Husbandry, 1933."

Both birds were placed under the experimental conditions of housing and feeding on October 13, 1934, and bird 1 was brought into the balance experiment on November 29. Bird 2 was brought into the balance experiment on March 7, 1935. Bird 1 laid 26 eggs from October 13 to November 29 and bird 2 produced 88 eggs from October 13 to March 7.

METABOLISM CAGES

The birds were housed in wire cages 18 inches square, 18 inches high at the back and 21 inches high at the front. The floors consisted of strong wire mesh with an upward slope from front to back which enabled any eggs laid to roll down into a safe receptacle extending for three inches beyond the front of the cage. The cages were supported on a wodden framework and were fitted up with strong glass dropping trays for the collection of the excreta. The front of the cage was fitted so that the birds could have easy access to food and water. Mash was fed in a galvanized container fixed just outside the front of the cage and every precaution was taken to prevent loss of food. Throughout the experiment the only losses were due to material adhering to the birds' beaks. This material was collected with the excreta. Grain was fed in small galvanized feeding troughs inside the pens. Losses of grain were also collected with the faeces.

RATIONS

The birds were fed the following mash and grain rations from October 13 to the end of the experiment:

Mash		*Grain*	
Bran	20	Maize	1
Weatings	30	Wheat	1
Yellow Maize Meal	20	Oats	1
Sussex Ground Oats	10		
Alfalfa Meal	10		
Cod Liver Oil	2		
Salt	1		

The only other supplement was oyster shell supplied ad libitum.

The rations were mixed by hand and at each mixing enough food was made up to last for a period of three weeks.

FEEDING AND SAMPLING TECHNIC

The birds were given a weighed quantity of mash each week, the residual food being collected and weighed at the same time of day the following week. The weekly mash consumption was calculated by subtracting the residue from the amount fed. The birds were given about one-half ounce of grain at 7 AM every morning and a larger quantity of grain each evening according to appetite. The weekly quantity of grain consumed was obtained by weighing out a given quantity every week and subtracting the residue at the end of each week.

Samples of food were taken from bulk from each mixing and assays were made of the total nitrogen content.

The excreta were collected every morning at 7 AM and stored in a glass container at a temperature of approximately 0°C. The total amount of excreta collected weekly was used for determination of the nitrogen. The eggs were collected daily and all the eggs laid during each period were analysed for their nitrogen content.

ANALYTICAL TECHNIC

Preliminary experiments on the estimation of nitrogen in the whole egg showed that digestion required large amounts of sulphuric acid and that the whole operation occupied several hours. Estimation of the nitrogen content of the whole egg had a further disadvantage in that no check could be made on the accuracy of the estimation.

It was finally decided to use the following method of estimation of nitrogen in the eggs. The eggs laid by the birds during each weekly period were broken and the contents run into a beaker. The con-

tents were weighed and thoroughly beaten with an ordinary egg beater. Two portions of 7-10 grams were then removed, weighed, treated with sulphuric acid and the nitrogen determined in the usual manner. If there were more than three eggs in any one period the number of eggs was divided as nearly as possible into halves and duplicate determinations were carried out on each half.

The shells were roughly crushed up, put into a Kjeldahl flask, treated with sulphuric acid and the nitrogen determined by the Kjeldahl method.

The faeces collected each week were weighed, thoroughly mixed and crushed in a large motar and triplicate portions weighed out for analysis. The whole operation was carried out as rapidly as possible in order to avoid error through loss of weight from evaporation.

The total nitrogen consumed in the mash and grain was determined by the Kjeldahl method.

RESULTS

Tables 1 and 2 give the body weight, mash and grain consumed, the number of eggs laid, the weight of the eggs produced and the weight of faeces excreted for birds 1 and 2 respectively during each week of the experiment.

TABLE 1.—*Bird 1 (28 weeks)*

Week	Wt. of bird	Wt. of mash	Wt. of grain	Wt. of faeces	No. of eggs	Wt. of eggs
	gm.	gm.	gm.	gm.		gm.
0	2778					
1	2878	227	454	1531	4	229.4
2	2778	510	454	1585	5	297.3
3	2764	510	454	1225	4	228.6
4	2778	397	454	1215	4	240.0
5	2849	397	454	1315	4	299.3
6	2821	439	454	1030	5	300.1
7	2821	510	454	1300	4	232.7
8	2934	539	454	1400	4	240.1
9	2991	312	142	1260	4	240.6
10	3005	539	576	1255	4	243.3
11	3048	454	397	1150	4	244.5
12	2991	454	454	1390	4	244.8
13	2948	454	425	1310	5	306.7
14	3033	454	397	1571	3	182.6
15	3033	510	397	1512	5	311.9
16	3118	454	312	1610	3	182.1
17	3033	425	454	1430	6	368.8
18	3005	397	425	1045	4	242.8
19	3019	539	454	1205	5	304.6
20	2977	510	454	1390	6	361.8
21	2920	510	454	1778	4	250.0
22	2807	657	454	1243	7	415.5
23	2807	652	312	1258	4	228.3
24	2892	567	397	885	2	120.0
25	2863	510	369	1225	5	306.1
26	2835	680	255	1298	5	303.6
27	2807	652	425	1223	4	244.4
28	2835	624	425	1168	5	307.1

Tables 3 and 4 show the amount of nitrogen consumed in the mash and in the grain, the total amount consumed, the nitrogen in the eggs and voided in the faeces, the total nitrogen excreted and the nitrogen balance for each period of one week.

TABLE 2.—*Bird 2 (13 weeks)*

Week	Wt. of bird	Wt. of mash	Wt. of grain	Wt. of faeces	No. of eggs	Wt. of eggs
	gm.	gm.	gm.	gm.		gm.
0	2807					
1	2863	624	425	1750	5	302.1
2	2948	624	340	1921	5	290.3
3	2807	567	397	1620	6	354.6
4	2778	510	454	1222	5	298.7
5	2778	624	454	1460	5	299.5
6	2807	737	454	1570	6	360.3
7	2792	737	454	1720	5	302.1
8	2772	879	454	1450	3	178.7
9	2778	794	454	1160	5	303.8
10	2807	539	425	927	4	243.4
11	2807	567	369	1162	3	191.1
12	2835	680	340	1175	3	185.6
13	2863	567	454	1035	4	252.9

DISCUSSION OF RESULTS

The appetite of both birds was very satisfactory throughout the whole experiment but there were considerable variations in food consumption from week to week, especially in the case of bird 1. The food consumption per week for bird 1 ranged from 454 gm. to 1,115 gm. with an average weekly consumption for the whole period of 907 gm. or 130 gm. (4.6 oz.) per day.

TABLE 3.—*Bird 1 (28 weeks)*

Week	Nitrogen in mash	Nitrogen in grain	Total nitrogen consumed	Nitrogen in eggs	Nitrogen in faeces	Total nitrogen excreted	Balance
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	5.07	7.47	12.54	4.37	13.63	18.00	−5.46
2	11.40	7.47	18.87	5.35	13.63	18.98	−0.11
3	11.40	7.47	18.87	4.09	10.54	14.63	+4.24
4	8.87	7.47	16.34	4.14	10.45	14.59	+1.75
5	8.87	7.47	16.34	4.29	13.00	17.29	−0.95
6	9.50	7.47	16.97	5.65	11.33	16.98	−0.01
7	10.79	7.29	18.08	4.13	11.83	15.96	+2.12
8	11.41	7.29	18.70	4.28	12.74	17.02	+1.68
9	6.59	2.29	8.88	4.57	12.60	17.17	−8.29
10	11.41	9.16	20.57	4.05	12.80	16.85	+3.72
11	9.60	6.38	15.98	4.48	11.27	15.75	+0.23
12	9.60	7.29	16.89	4.39	11.95	16.34	+0.55
13	9.60	6.87	16.47	5.51	12.45	17.96	−1.49
14	9.60	6.38	15.98	3.21	13.66	16.87	−0.89
15	10.79	6.38	17.17	6.08	12.53	18.61	−1.44
16	9.60	5.01	14.61	3.40	13.36	16.76	−2.15
17	5.99	7.29	16.28	6.95	13.73	20.68	−4.40
18	8.41	6.85	15.26	4.41	11.90	16.31	−1.05
19	11.39	7.29	18.68	5.59	11.63	17.22	+1.46
20	10.81	7.30	18.11	6.72	12.22	18.94	−0.83
21	10.81	7.30	18.11	4.54	14.53	19.07	−0.96
22	13.76	7.30	21.06	7.81	15.36	23.18	−2.12
23	13.82	5.01	18.83	4.63	13.49	18.12	+0.71
24	12.36	6.03	18.39	2.24	11.62	13.86	+4.53
25	11.13	5.60	16.73	5.54	12.76	18.30	−1.57
26	14.84	3.88	18.72	5.69	12.95	18.64	+0.80
27	14.22	6.46	20.68	4.45	11.15	15.60	+5.08
28	13.60	6.46	20.06	5.76	11.98	17.74	+2.32

The food consumption per week for bird 2 ranged from 936 gm. to 1,333 gm. with an average weekly consumption for the period of 1,072 gm. or 153 gm. (5.4 oz.) per day.

The weights of the birds fluctuated from week to week but throughout the whole period both birds handled very well and maintained good body condition. Bird 1 weighed 2,778 gm. (98 oz.) at the commencement and 2,835 gm. (100 oz.) at the end of the experiment, a gain of 57 gm. (2 oz.) for the whole period. Bird 2 weighed 2,807 gm. (99 oz.) at the commencement of the experiment and 2,863 gm. (101 oz.) at the end of 13 weeks, a gain of 56 gm. (2 oz.) for the whole period.

In the case of both birds the nitrogen consumption varied considerably from period to period. The nitrogen consumption per week for bird 1 varied from 8.8 gm. to 21.06 gm. with an average of 17.29 gm. or 2.47 gm. per day. The nitrogen consumption per week for bird 2 varied from 17.96 gm. to 25.85 gm. with an average of 20.53 gm. or 2.93 gm. per day. In the balance experiment carried out by Willcox (1934) the average nitrogen consumption per day was considerably higher, bird 1 consuming 3.54 gm. and bird 2 consuming 3.21 gm.

The amount of nitrogen excreted in the faeces varied considerably from week to week, the figures for bird 1 ranging from 10.45 to 15.36 gm. and for bird 2 from 11.35 to 15.07 gm. It will be observed that the amount of nitrogen excreted in the faeces bears no close relationship to the consumption of nitrogen during each period. In this respect the results do not correspond with those obtained by Willcox who obtained a fairly close relationship between the nitrogen consumed and the amount retained for egg production. The nitrogen balance for both birds fluctuated considerably from week to week. During period 9 bird 1 lost 8.29 gm. of nitrogen whereas in period 27 a positive balance of 5.08 gm. nitrogen was obtained. The negative balance of 8.29 gm. nitrogen, whicb may be regarded as abnormal, was entirely due to poor food consumption. During that period the consumption of nitrogen was only 8.88 gm. It is interesting to note that the production of four eggs was quite normal for the period. The largest negative nitrogen balance with bird 2, of 3.62 gm. was shown during the third period. During period 8 a positive nitrogen balance of 9.38 gm. was obtained. This can only be explained by the high consumption of 25.85 gm. nitrogen and a slightly lower than average egg pro-

TABLE 4.—*Bird 2 (13 weeks)*

Week	Nitrogen in mash	Nitrogen in grain	Total nitrogen consumed	Nitrogen in eggs	Nitrogen in faeces	Total nitrogen excreted	Balance
	gm.	gm.	gm.	gm.	gm.	gm.	gm.
1	13.18	6.87	20.05	6.00	14.00	20.00	+0.05
2	13.18	5.47	18.65	5.72	14.21	19.93	−1.28
3	11.99	6.38	18.37	6.92	15.07	21.99	−3.62
4	10.79	7.29	18.08	5.78	13.37	19.15	−1.07
5	13.18	7.29	20.47	5.95	13.62	19.57	+0.90
6	15.63	7.30	22.93	7.04	14.33	21.37	+1.56
7	15.63	7.30	22.93	6.02	13.72	19.74	+3.19
8	18.55	7.30	25.85	3.54	12.93	16.47	+9.38
9	16.83	7.30	24.13	5.93	13.94	19.87	+4.26
10	11.74	6.46	18.20	4.94	12.68	17.62	+0.58
11	12.36	5.60	17.96	3.63	12.95	16.58	+1.38
12	14.84	5.17	20.01	5.01	14.01	17.74	+2.27
13	12.36	6.90	19.26	4.82	11.35	16.36	+2.90

duction for the period, (three eggs).

A study of the figures shows clearly that the negative balances with both birds arose during periods of low food consumption. In the case of bird 1 a negative balance was obtained in 15 periods, and in 11 of these the food consumption was less than the average weekly consumption of 17.29 gm. nitrogen. In three of the remaining four periods the food consumption was just above the average. The remaining negative balance was obtained during period 22 when seven eggs were produced and the maximum amount of 15.36 gm. nitrogen was excreted in the faeces. In the case of bird 2 negative balances were only obtained on three occasions. On each of these occasions the nitrogen intake was lower than the average weekly nitrogen intake of 20.53 gm.

For the whole period of 28 weeks bird 1 showed a negative nitrogen balance of 3.25 gm. Bird 2, on the other hand, showed a positive nitrogen balance of 20.50 gm. for the experimental period of 13 weeks. The combined results for both birds suggest that the ration employed in the experiment supplied sufficient nitrogen to maintain good production without loss of body nitrogen.

The figures indicate that there is not necessarily a storage of nitrogen prior to heavy production, for in period 21, bird 1 had a negative nitrogen balance of 0.96 gm. but laid seven eggs in period 22.

This is in agreement with the findings of Halnan and of Willcox, that the necessary protein for egg production can be drawn largely, if not directly, from the food consumed during the period of production. The figures show further that the nitrogen balance during any period does not depend on the number of eggs laid but on the amount of nitrogen consumed in the food. There is no suggestion in this experiment that period of high egg production is accompanied by a better utilization of the nitrogen consumed. Egg production was satisfactory throughout the experiment, the eggs laid being of average size and of normal shell texture and candling appearance. During the period of 28 weeks bird 1 produced 123 eggs (average production 63 percent), averaging 60.8 gm. in weight. During the 13 weeks bird 2 produced 59 eggs (average production 65 percent), averaging 60.4 gm. in weight.

UTILIZATION OF FOOD NITROGEN BY BIRDS 1 AND 2 FOR MAINTENANCE AND PRODUCTION

A digestibility trial carried out by Willcox (1934) on two Rhode Island Red hens, fed on a mash very similar to that used in the experiment, demonstrated that the digestibility coefficient of the nitrogen consumed was 80.95 percent. From a survey of digestibility trials carried out by various research workers (Jull, 1930) it can be safely assumed that with the rations used in this experiment the nitrogen digestibility coefficient of the combined mash and grain fed did not exceed 80 percent.

During the course of the experiment bird 1 consumed 484.17 gm. nitrogen, voided 351.09 gm. nitrogen in her faeces, and produced 123 eggs containing 136.33 gm. of nitrogen. The nitrogen balance for the whole period was 3.25 gm. Assuming that the food nitrogen was digested to the extent of 80 percent, the amount of nitrogen digested was 387.34 gm. With a negative balance of 3.25 gm. nitrogen, 390.59 gm. of digestible nitrogen was used by this bird for maintenance and the production of 123 eggs during a period of 28 weeks.

Bird 2 consumed 266.89 gm. nitrogen, voided 176.18 gm. nitrogen in her faeces, and produced 59 eggs containing 70.21 gm. of nitrogen. The nitrogen balance for the period was +20.5 gm. The amount of food nitrogen digested is estimated at 213.51 gm.

and since 20.5 gm. was stored 190.31 gm. of digestible nitrogen was used for maintenance and the production of 59 eggs over a period of 13 weeks.

The utilization of nitrogen for egg production can be obtained by subtracting the maintenance nitrogen requirements for egg production from the amount of available nitrogen for maintenance and production. Ackerson, Blish, and Mussehl (1923) measured the endogenous nitrogen metabolism of non-moulting Rhode Island Red hens and obtained an average daily loss of 144 mgm. per km. of body weight. The average body weights of birds 1 and 2 during the experiment were 102.6 oz. (2.909 kg.) and 99.2 oz. (2.812 kg.). On the basis of the above figures the average daily endogenous loss of nitrogen would be 418.9 and 404.9 mg. for birds 1 and 2. According to these standards the nitrogen requirements for bird 1 for a period of 196 days would be 82.10 gm. and for bird 2 over a period of 91 days 36.85 gm. By subtracting these figures from the amount of nitrogen available for maintenance and egg production it is possible to obtain the nitrogen requirements for egg production. Bird 1 required 308.49 gm. of digestible nitrogen to produce 136.33 gm. of egg nitrogen and bird 2 required 153.47 gm. to produce 70.21 gm. of egg nitrogen. These figures represent 2.26 and 2.19 gm. of digestible nitrogen per gm. of egg nitrogen produced by birds 1 and 2 respectively. These figures are appreciably lower than those obtained by Willcox, who obtained figures of 2.79 and 2.55 gm. of digestible nitrogen per gm. of egg nitrogen.

Bird 1 required 308.49 gm. of digestible nitrogen to produce 7,477 gm. of egg and bird 2 required 153.46 gm. of digestible nitrogen to produce 3,563.1 gm. of egg. These figures represent 2.34 and 2.44 gm. of digestible nitrogen per two ounce of egg. In terms of digestible protein these figures represent 14.63 and 15.25 gm. per two ounce egg for birds 1 and 2 respectively and are considerably higher than Halnan's figure of 10 gm. of digestible protein per two ounce of egg. It must be remembered, however, that these figures are overestimates of the protein requirements for egg production since they are obtained by taking the endogenous loss of nitrogen as the maintenance requirements. In actual practice the biological value of the proteins in the foods fed would be considerably lower than 100 for maintenance. In the absence of more complete information on the biological value of the proteins used it is not possible to arrive at more accurate figures than 2.26 and 2.19 gm. of digestible nitrogen per gm. of egg nitrogen. According to Halnan's standard, bird 1 laying 123 eggs, average weight 60.8 gm. over a period of 196 days would require 517.36 gm. of digestible nitrogen for maintenance and production. In actual practice this bird gained 2 oz. over the period and used 390.59 gm. of digestible nitrogen. Again, according to these standards, bird 2 laying 59 eggs, average weight 60.4 gm. over a period of 91 days would require 236.54 gm. of digestible nitrogen. During the period this bird actually gained 2 oz. in weight, and used only 190.31 gm. of digestible nitrogen.

In this experiment the rations fed gave satisfactory egg production even though they contained considerably less protein than current feeding standards. The foregoing estimates suggest that satisfactory maintenance and production can be provided by rations containing less protein than those generally adopted or recommended in feeding standards.

SUMMARY

This paper outlines the results obtained from a balance experiment carried out with

two Rhode Island Red pullets fed on a cereal plus mineral ration.

The variations observed in the nitrogen balance do not appear to be connected with the egg production, but rather with the appetite of the bird. A large food consumption over any one period usually resulted in a positive nitrogen balance. The results do not suggest that a high egg production during any period is preceded by a marked storage of nitrogen.

The results show that the nitrogen required for egg production can be drawn from the food supplied, even though the ration is considerably lower in protein than the accepted standards.

An estimate is made of the digestible nitrogen required by the birds during the experiment for maintenance and production and the figures show that maintenance and production can be supplied by rations containing considerably less digestible protein than the present accepted feeding standards.

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Transmission of Fowl Leukosis

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MARKED progress has been made during the past decade in determining the true nature of fowl leukosis. Most contributions, however, have been in the pathology of the disease and in experimental transmission. All of this information is necessary for a better knowledge of the fundamental nature of the disease, but so far the poultryman has received no definite clues regarding natural transmission within his flock, nor any method for control. The present investigations reported herein suggest at least two ways in which the disease may be disseminated within a flock that contains one or more leukotic fowls.

There has been written considerable about leukosis and allied conditions of fowls with no general agreement as to what specific conditions should come under this name. According to Dorland's medical dictionary, the term leukosis has two meanings: 1. An aggregation of dead or dying leukocytes seeking to escape from the body. 2. Leukemia. Neither of these definitions is entirely fulfilled in the expressions of this disease in fowls. Ellermann (1) was first to use the term "leucosis" in his study of leucemia problems. He used it to designate a transmissible leukemia-like disease of fowls in which erythrocytes, granulocytes, or lymphocytes may be involved. This conception distinguishes it from a true leukemia, but still fails to satisfy all the requirements of a term suitable for this disease. Many writers have used the term as proposed by Ellermann, others have spelled it with a "k." The term lymphomatosis has been used in referring to the lymphoid form, but, since all these various expressions of the disease have been produced by injection materials from any of the different forms, it seems that the term lymphoid leukosis is preferable. To place this disease-complex on a uniform basis so that the literature on this subject would be less easily confused, it is hoped that the term leukosis will be accepted, and that erythro, lymphoid, and myeloid will be used in its connection to designate the various forms.

The neurolymphomatosis described by Pappenheimer and his co-workers (3) appears to be an expression of the lymphoid form of the disease. Johnson (2) found that any of the various expressions, that is, erythro, lymphoid, and myeloid forms, may be produced by injections of bacteria-free filtrates of blood and other tissue extracts from leukotic fowls, regardless of the particular form had by the donor. This multiplicity of expressions of the disease-complex in response to what appears to be a single causative agent adds to the difficulty of obtaining reliable data on the occurrence of the disease under natural conditions.

Certain facts, deduced from the success with experimental inoculations in using blood and other tissue extracts, suggest that minute amounts of these materials are capable of causing the disease if they are injected directly into the blood of susceptible birds under proper conditions. These facts suggest, moreover, that the causative agent might be carried from an affected individual to well birds by certain blood-sucking parasites, or possibly by certain vaccination procedures in which small

amounts of infective material could be transferred from affected birds to well birds. The experiments herein reported indicate that leukosis may be transmitted from sick to well fowls by the common red mite (*Dermanyssus gallinae*), and also through the common procedure of vaccination for the prevention of chicken pox in fowls.

REVIEW OF LITERATURE

On examination of the literature one is impressed with the scarcity of material on this phase of the leukosis problem. With the exception of the report by Ratcliffe and Stubbs (5), who used mosquitoes (*Culex pipiens* and *Aedes egypti*) and mites (*Dermanyssus gallinae*) in attempts to transmit fowl leukosis from sick to well birds, apparently very little work has been done to determine the role played by vectors in transmission of the disease. In their report they mention that Anderson and Bang failed to transmit leukemia by mites. No details of this work, however, are reported. These workers also state that Jármai has reported a case of leukosis among chickens infested with the ticks (*Argas*) that had had an opportunity to feed on infected fowls.

No reference has been found in the literature to work on the role of vaccination in transmission of fowl leukosis.

MATERIALS AND METHODS

The experiments in which mites were engaged involved the use of 16 six-week-old White Leghorn chickens obtained from eggs hatched in our laboratories. All eggs came from a flock in which there had been no case of the disease for the past four years. The chicks obtained were immediately placed in sterile metal cages with raised wire floors, and kept in these throughout the experiments. The vaccination experiments involved the use of 38 similar birds. In each case an equal number of birds from the same source were used as controls, making a total of 108 birds in all. Separate rooms for the treated groups of birds, and for the controls, as well as individual caretakers for each room, were provided to eliminate any possibility of direct or indirect contact between the exposed and control groups of birds.

Various ways for exposing birds to mites were considered. A preliminary study convinced us of the importance of knowing definitely that the mites were engorged with blood of a sick bird before placing them upon the well birds. The habits of these mites of feeding upon birds only at night and withdrawing to cracks and crevices in the day time, along with their markedly red and engorged appearance after feeding, make it possible to determine their role in transmission of the disease with considerable precision. The method used consisted in collecting the mites in glass vials and keeping them under observation for several days until they were entirely gray in appearance and completely free from blood obtained from any other fowls. A leukotic bird was then placed in a large stone crock with perfectly smooth surfaces interiorly. The crock containing the leukotic bird was placed in a dark room and several mites, 20 or more, were placed on the bird. Two wooden blocks, containing several small grooves made by sawing into the blocks to a depth of about one-eight inch (Figures 1 and 2), placed in apposition and held with rubber bands, were then placed beside the bird for a period of from 12 to 18 hours. At the expiration of this interval lights were turned on for an hour or more, or until the mites had gone into the crevices of the blocks. The blocks could be separated sufficiently to count the number of mites and to note that they were engorged with blood. These blocks, containing the engorged mites, were then placed in a sterile cage containing the susceptible bird to be

fed upon, and left for two nights to make sure that they had attacked the bird. At this time the mites were destroyed and the bird placed in a sterile cage for observation.

The follicle method of vaccination was used. This consists chiefly in plucking approximately a dozen feathers from the lateral surface of the leg just dorsal to the hock joint, or tibio-metatarsal articulation, and rubbing the vaccine into the follicles with a stiff bristle brush.

EXPERIMENTS WITH MITES

Experiment 1. In this experiment four birds approximately two months of age were infested with mites that had engorged themselves on blood from a chicken affected with myeloid leukosis. The affected bird, on autopsy immediately after the mites had fed, was found to have an enlarged liver and spleen. The radial marrow was pink in color and of a gelatinous consistency. Stained sections revealed active areas of ectopic granulocyte production in the liver and spleen. On examination of stained blood smears the various cells were found to occur proportionately as follows: Granulocytes with eosinophilic rod granules 37, Granulocytes with eosinophilic round granules 13, Basophils 1, Monocytes 3, Lymphocytes 1, Hemocytoblasts 2, and Myelocytes 33. On the basis of these findings this is considered to be a case of the myeloid form. Of the four birds that were bitten by mites that previously had fed on this bird with myeloid leukosis, one bird developed the lymphoid form of the disease. The bird was killed and autopsied 132 days following the attack by mites. Extensive proliferation of hemocytoblasts and lymphocytes were found in the liver and sciatic nerves.

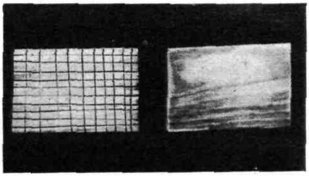

FIG. 1

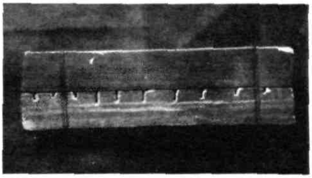

FIG. 2

Experiment 2. In this experiment six birds similar to those used in experiment 1, were infested with mites that were engorged with blood obtained from a bird affected with lymphoid leukosis. Autopsy of this bird immediately after the mites had been transferred revealed slight enlargement of liver, spleen, and right sciatic nerve. On examination of stained sections from these organs extensive proliferations of hemocytoblasts and lymphocytes were found. Blood smears from this bird, on the day that the mites had fed, revealed the following percentages of cells: Granulocytes with eosinophilic rods 9, granulocytes with eosinophilic round granules 0, Basophils 3, Monocytes 0, Lymphocytes 85, Hemocytoblasts 11, Myelocytes 2. From these findings this is considered to be a case of lymphoid leukosis. Of the six birds that were infested with mites that had fed on this bird, two developed lymphoid leukosis and one myeloid leukosis. The diagnoses were based on lesions as described for these forms.

Experiment 3. Six birds, similar to those used in the previous experiments, were in-

fested with mites that had engorged on blood from a bird with the lymphoid form of the disease. This bird was paralyzed, and the autopsy revealed that it was typical of the neurolymphomatosis (Pappenheimer et al). One of the six birds developed symptoms and lesions very similar to those of the donor 284 days following infestation with the mites.

All birds in these experiments that did not develop symptoms of disease were kept for more than a year before they were destroyed. The earliest case to develop occurred 85 days after infestation with mites.

The control birds were kept under identical conditions, and no clinical case of leukosis developed among these. These birds were all killed and autospied at the close of the experiment, which was more than 12 months since the first birds were infested with mites. The autopsies revealed no case of incipient leukosis among the controls.

EXPERIMENTS WITH VACCINATION

Experiment 1. The first vaccination experiment was undertaken on February 23, 1935. In this experiment 19 10-week-old White Leghorn birds were vaccinated by the follicle method in the usual manner, with pigeon-pox vaccine. Immediately before vaccinating this group of birds a leukotic fowl was vaccinated in the usual manner, and the brush used for vaccinating this bird was returned to the vaccine vial to be used for vaccination of the 19 hitherto unexposed birds. The leukotic bird, on autopsy immediately after the vaccination, proved to be an incipient case of hemocytoblastosis. The peripheral blood contained numerous immature lymphoid cells (hemocytoblasts), the radial marrow was hyperplastic, and there were marked periportal proliferations of lymphoid-like cells in the liver.

The birds that did not develop leukosis were kept for more than a year after vaccination, at which time they were killed and autopsied to determine their condition. Those that developed the disease either died or were killed and autopsied at that time to determine the type of disease present.

In this group five of the 19 vaccinated birds developed a form of the disease. Three of these were interpreted as lymphoid forms, one as hemocytoblastosis, and one as a combination of hemocytoblastosis and erythroleukosis.

Experiment 2. In this experiment eight birds were vaccinated with chicken-pox vaccine (follicle method) on January 4, 1936. Just previously, a leukotic fowl had been vaccinated and the brush used for vaccination returned to the vaccine vial, to be used on this group of birds. This leukotic fowl, on autopsy after the vaccination, was considered to be affected with lymphoid leukosis. One leg was paralyzed. The sciatic nerve of this leg was greatly enlarged, edematous, and of a brownish color with loss of normal striations. The liver and spleen were at least twice as large as the normal for this bird. The radius marrow was red in color and gelatinous in consistency.

In this group only one of the vaccinated birds developed leukosis. This was a case of hemocytoblastosis. One of the controls for this group developed myeloid leukosis.

Experiment 3. In this experiment 11 birds were vaccinated with pigeon-pox vaccine (follicle method), in the usual manner, on February 25, 1936. A bird with myeloid leukosis was vaccinated immediately before and the brush used for this vaccination was returned to the vaccine vial to be used in vaccinating the 11 susceptible birds. These birds were kept for a year following the vaccination. Only one bird developed lesions. This bird developed a small tumor (2 x 2 cms.) in the pectoral muscles. On section this proved to be a fibrosarcoma. The radius marrow was hyperplastic. Around the tumor there were several areas of proliferating

hemocytoblasts. This may be also considered as an expression of leukosis. All of the control birds for this group have remained negative.

DISCUSSION AND CONCLUSIONS

As regards the experiments with the transmission of leukosis with mites, interest lies chiefly in the fact that this is the first time that evidence has been presented to indicate that such might well be a factor in dissemination of the disease in flocks that have one or more leukotic fowls, and where these mites are present. From the work of Ratcliffe and Stubbs (5) with mosquitoes it appears that the etiologic agent of leukosis remains viable in the bodies of mosquitoes (*Culex pipiens* and *Aedes egypti*) for less than 24 hours. On the basis of the results in the experiments with mites (*Dermanyssus gallinae*), reported herein, it appears that the leukosis agent must remain viable in the bodies of these for at least 12 hours. This deduction is based on the observation that these mites usually do not attack a second bird immediately after completely engorging themselves on blood from a fowl. They usually wait until the following night to attack a second bird unless they are disturbed, in which case they may complete their engorgement immediately on a second fowl. These experiments, therefore, do not explain whether these mites are biological-intermediary hosts to the leukosis agent, or simply act as mechanical carriers. It is conceivable that enough infective material could get into the secretions of the mites so that it might be injected into the bird at the time the mite was engorging on its blood. Neither does this work enlighten us as to the extent that leukosis might be disseminated by mites under natural conditions.

At the present time most progressive poultrymen make an attempt to keep their birds free from mites. Just how successful they are in accomplishing this is difficult to judge, as it is well known that these mites hide in obscure places during the day and come out to feed on the birds while they are on the roosts at night. Unless the poultryman is careful in examining crevices and other places that might serve as hiding places for these mites he cannot be sure as to the extent of damage that they might cause. Undoubtedly, this might be an important factor on farms where no attempt is made to get rid of these mites.

As regards the vaccination experiments, one may at once reason that this is perhaps a very important means of spreading leukosis within a flock that contains one or more leukotic fowls. An analysis of the three experiments carried out indicates that leukosis in the incipient stages is most readily transferred in the vaccination process, since five cases occurred among the 19 birds vaccinated following the vaccination of a bird with an early case of hemocytoblastosis, and only one case occurred in each of the other groups. The tendency toward extensive vaccination for the prevention of chicken pox, with practically no regard for the danger of spreading disease within a flock by this procedure, may be one explanation for the increased number of cases of leukosis in recent years.

With this evidence to indicate that leukosis may be spread from an affected bird to several well birds in a flock by the vaccination procedure, many problems arise for consideration. First, it should be determined how important this avenue of transmission is on a large scale. Second, it should be determined whether the stick method or follicle method of vaccination is the greater factor in dissemination of the disease. Third, emphasis might be placed upon the purity of the vaccines used. Recently, attention has been called to certain localities in which the flock owners make their own chicken-pox vaccines. These are made roughly by scarifying the combs of cock-

erels, whose status of health has not been ascertained, and rubbing on some virus from unknown source as far as other diseases are concerned. After two weeks, or when the scabs are well developed, they are removed, dried, ground up, and used to vaccinate, not only their own flocks, but their neighbors' as well. In these sections it has been noted that an increasing number of cases of leukosis have developed. With this meager information on the subject one would be justified in urging the importance of using only chicken-pox vaccine derived from birds whose health status has been determined by qualified individuals.

The transmission of leukosis in these experiments, both by mites and by the vaccination procedure, bring further support to the work of Ellermann (1), Patterson, et al (4), Johnson (2), as well as others, that any of the various expressions may develop regardless of the particular manifestation of the disease had by the donor of the disease producing agent.

SUMMARY

Evidence obtained in these experiments compels one to conclude that mites (*Dermanyssus gallinae*) are capable of transmitting the leukosis agent from an affected bird to well birds under conditions that lead one to believe that this might take place under the conditions that poultry are managed at the present time.

The vaccination experiments compel the conclusion that the leukosis agent may be transferred mechanically from a leukotic bird to several well birds in a flock by the common procedure of vaccination to prevent chicken pox in fowls.

The data obtained in these experiments should tend to stress the importance of further investigations along these lines that might furnish means for prevention of the dissemination of this widespread disease.

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The Alleged Intoxication Factor in Fish Meal

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IT HAS been stated by Cook and Scott (1935a, b) that a condition, the main symptoms of which were (1) severe anemia, (2) increased clotting time of blood, (3) distortion of the blood picture with appearance of immature red cells and great increase in white cells, and (4) numerous hemorrhages, was noted in chicks given a diet of yellow corn 64, wheat 20, "functionally-low-sulphur fish meal" 10, dried yeast 1, calcium carbonate 1.1, tri-calcium phosphate 0.4, and sardine or cod liver oil 1. When the above fish meal was replaced by "functionally-high-sulphur fish meal" the symptoms did not occur. The symptoms were attributed to the presence of nitrogenous bases which in the case of the latter fish meal were supposedly detoxified by the sulphur compounds. The term "functionally" was not further explained. It is evident that the diet was deficient in the anti-hemorrhagic vitamin, which Dam (1935a, b) and Almquist and Stokstad (1935a, b) have demonstrated to be required by the chick for normal blood clotting time and the prevention of hemorrhage.

In a later paper Scott and Cook (1936) have reported that nitrogenous bases present in poultry diets containing fish meal are the cause of an intoxication syndrome consisting of slightly prolonged blood clotting time, slight hemorrhages, non-hemorrhagic anemia, and greatly distorted blood picture including profound increase in number of leucocytes. The same syndrome was reported in the case of diets not containing fish meal but supplemented with various kinds of amines, including trimethylamine, a substance known to occur in fish meals. This syndrome was noted in spite of the presence of vitamin K (anti-hemorrhagic vitamin) in the diets.

In view of the facts that fish meal is used widely in commercial poultry feeding, that we have employed fish meal in diets for our work on the anti-hemorrhagic vitamin, and that the paper by Scott and Cook creates a very serious issue as to the advisability of feeding fish meal to poultry, we have felt it necessary to conduct further studies on the reported toxicity of fish meal.

METHODS AND RESULTS

Our first series of experiments were designed to test the effect on blood characteristics of practical diets such as those widely used for rearing chicks. when such diets were supplemented with commercial casein as compared to a standard feeding grade of sardine meal. In addition, casein diets containg added trimethylamine hydrochloride or amines from sardine meal were studied.

White Leghorn chicks were placed in wire floored, all metal, electrically heated battery brooders and given the experimental diets at one day of age. They were kept exclusively on these diets for eight weeks.

Blood clotting time was determined by puncture of a main wing vein, allowing about 0.2 c.c. of blood to fall into a clean glass vial and placing the vial in a shaking mechanism in a water thermostat held at 35°C. Time of puncture of the wing vein and the time at which the blood sample clotted was noted, and the difference taken as the clotting time. When this clotting time

was greater than 30 minutes, the sample was considered negative for practical purposes.

Hemoglobin was determined on another sample of blood by the Wu alkali hematin method. Blood samples were compared by colorimeter with a sample of similarly treated chicken blood in which the hemoglobin level had been determined by the oxygen capacity method.

Counts were made of total white cells and total red cells on the same sample of blood by diluting 1 to 200 with Toisson's fluid and using a standard counting chamber.

Amines were extracted from a commercial feeding brand of sardine meal by five treatments with several volumes of hot 95 percent ethanol containing hydrochloric acid at a strength of 0.1N. The alcoholic extracts were concentrated under vacuum, taking precautions to maintain acidity, then made strongly alkaline with sodium hydroxide and distilled at atmospheric pressure into dilute sulphuric acid. For example, we obtained from 1 kilogram of sardine meal, volatile bases equivalent to 26.9 c.c. of 0.5N acid. Removal of ammonia was effected by shaking with yellow mercuric oxide leaving bases equivalent to 13.4 c.c. of 0.5N acid. These bases calculated to trimethylamine correspond to .0396 percent of the fish meal.

It is necessary at this point to call attention to an error in the paper of Scott and Cook in regard to the assumed behavior of trimethylamine oxide. They state that if such amine oxides exist in the fish meal they are not volatile when fish meal is boiled in water and made alkaline by magnesium oxide. We obtained a sample of pure trimethylamine oxide[1] and found that, in fact, it was not volatile in the presence of magnesium oxide alone and yielded no trace of bases distillable into standard acid. However, in the presence of fish meal and magnesium oxide, the amine oxide was rapidly and quantitatively reduced to volatile amines and recovered as such, in addition to the nitrogenous bases from the fish meal itself. The total bases found by Scott and Cook with this procedure must, therefore, have included all or a large part of the amine oxides that may have been present, and the assumption that the fish meal may have contained considerable quantities of amine oxide, as well as the volatile bases found, is unjustified.

The diets used in our first experiment con-

TABLE 1.—*Diets, growth, mortality, hemoglobin levels, white cell counts, red cell counts, and blood clotting time in chicks eight weeks of age*

Diet	A	B	C	D
Composition of diet				
Basal Mixture	88½	88½	88½	88½
Sardine meal, standard feeding grade	12	—	—	—
Casein, common	—	9½	9½	9½
Bone meal	½	2	2	2
Trimethylamine hydrochloride	—	—	¼	—
Amine extract of sardine meal	—	—	—	20 (equivalent)
Total	101	100	100¼	100
Protein level	19.5%	20.4%		
Number of birds started	20	20	20	20
Average body weight, grams	449	416	294	351
Mortality, number of birds	2	3	1	1
Average blood clotting time, minutes	2.01	3.04	2.78	2.57
Average hemoglobin level, grams per 100 c.c.	8.44	8.38	7.97	7.93
Average white cell count $\times 10^3$	15.4	16.5	15.3	18.0
Average red cell count $\times 10^6$	2.08	2.33	2.18	2.21

[1] Kindly furnished by Professor T. D. Stewart.

tained in all cases: 5 parts dried alfalfa, 5 dried skimmilk, 15 wheat bran, 15 ground wheat, 30 ground yellow corn, 15 ground barley, 1 sardine oil, 2 ground limestone, and 0.5 salt. This basal mixture was supplemented with other ingredients which are listed in Table I, together with the experimental results.

Inspection of these results leads to the following conclusions.

1. Diet A, which corresponds very closely to chick mash formulas recommended by this division and which contained a standard poultry feeding brand of sardine meal, produced results which were not appreciably different from those obtained on a similar diet (B) in which casein replaced the fish meal. All phases of blood characteristics may be regarded as identical and normal. There is, therefore, no evidence of the "intoxication factor" in the fish meal used.

2. The addition of amines to the diet (C and D) produced no noticeable effect on blood composition, even at the comparatively high level of 0.25 percent of trimethylamine hydrochloride. The only appreciable effect noted was that on growth and probably this effect may be attributed to unpalatability of the diet as logically as to any influence. The feed consumption of birds on these diets was considerably below that of birds on diets A and B.

3. Mortality was not greater in any case than the usual early mortality in chicks which is almost invariably encountered both practically and experimentally.

Following the first series of experiments with practical diets, we next attempted to reproduce the results of Scott and Cook (1936) using several of their diets. The diets chosen were those given in Table 1 of their paper, namely 2A, 3C, and 4A, together with a similar diet corrected for certain probable water-soluble vitamin deficiencies.

Results reported by Scott and Cook with these diets were as follows:

2A: sardine meal, 20 percent. Lacked anti-hemorrhagic vitamin but contained amines present in sardine meal. Profound increase in leucocyte (white cell) count, anemia and prolonged blood clotting time.

3C. casein 15 percent. Normal diet. No amines present. Anti-hemorrhagic vitamin present. Normal blood characteristics.

4A: sardine meal 20 percent. Contained amines present in sardine meal. Anti-hemorrhagic vitamin present. Profound leucocytosis and anemia, attributed to nitrogenous bases.

Results obtained in our experiments with these diets are given in Table 2.

From these results several conclusions seem warranted:

1. Diets 2A and 4A produced no evidence of leucocytosis or of anemia. Diet 2A, lacking in the anti-hemorrhagic vitamin, produced a delayed blood clotting time, six of the birds on this diet having blood which failed to clot within 30 minutes and the remaining birds showing slightly prolonged blood clotting time. Diet 4A contained the anti-hemorrhagic vitamin as provided by dried alfalfa and produced normal blood clotting time. A few birds on diet 2A showed subcutaneous hemorrhage. Birds on diet 4A gave no slightest indication of hemorrhage.

2. Diet 3C, "normal" diet, produced distinctly inferior growth which may be attributed to lack of water-soluble growth factors. Aside from poor growth, no other abnormalities were found.

3. Modified diet. This diet was made up to correct the growth factor deficiencies which were apparent in the other diets from inspection of their formulas. Sufficient dried alfalfa was added to meet the requirements for the anti-hemorrhagic vitamin. Five percent of yeast was also added to supply adequate amounts of water-soluble vitamins. In our experience, less than 3 percent of a high-grade pure brewer's yeast has not been sufficient for this purpose in such diets. The growth obtained with this modified diet was

definitely greater than that obtained with the other diets. The next best growth was obtained with diet 2A containing 2 percent of yeast. It may be remarked at this point that Scott and Cook (1936) have also reported considerable amounts of amines in yeast.

4. There were no "profound" or even statistically significant differences in blood composition between any of the groups.

Several birds in these groups became so badly crippled because of slipped tendon that they were destroyed. Such birds are not shown in the mortality data. This occurrence of slipped tendon was expected because of the high phosphorus content of these diets.

TABLE 2.—*Diets, growth, mortality, hemoglobin levels, white cell counts, red cell counts, and blood clotting time in chicks eight weeks of age*

Diet	2A	3C	4A	Modified Diet
Composition of diet				
Casein, common	—	15	—	—
Sardine meal, standard grade	20	—	20	20
Alfalfa, dried	—	5	5	1
Brewer's yeast, dried	2	—	—	5
Calcium carbonate	1	1	1	1
Tricalcium phosphate	0.4	1	0.4	—
Sodium chloride	0.5	0.5	0.5	0.5
Cod liver oil	0.5	0.5	0.5	0.5
Ground yellow corn	to 100	to 100	to 100	to 100
Number of birds started	14	15	17	12
Average body weight, grams	398	250	371	448
Mortality, number of birds	2	0	0	0
Average blood clotting time, minutes	11.1[1]	1.73	2.07	1.54
Average hemoglobin level, grams per 100 cc.	7.39	7.44	7.34	8.66
Average white cell count, $\times 10^3$	13.7	13.7	13.0	21.3
Average red cell count, $\times 10^6$	1.64	2.11	2.03	1.94

[1] Average clotting time for 6 birds. Blood of the remaining birds failed to clot within 30 minutes.

DISCUSSION

The results reported above are so at variance with those of Scott and Cook that we are unable to account for the disagreement. The grade of fish meal used is not stated in their paper. They also do not give explicit information on growth and mortality of their birds but they do imply that mortality was severe when sardine meal was used. We have had experience with sardine meals of poor quality which did cause excessive mortality. This, however, is far from the case when standard feeding grades are used, either practically or experimentally, in otherwise good diets.

The age at which our birds were examined (eight weeks) should have allowed sufficient time for the diets to exert their full effect. The age of the birds used by Scott and Cook was not given but the statement was made that the birds were examined at ages up to 10 weeks.

Our results on the addition of trimethylamine hydrochloride to a casein diet failed to produce evidence of any departure of the blood picture from normal. We have not repeated any of the work of Scott and Cook with other types of amines which, in most cases, were fed in relatively massive doses.

As partial proof of their hypothesis, Scott and Cook have reinterpreted data from one of our papers (Almquist and Stokstad, 1935b) on the comparative effects of casein and sardine meal in diets deficient in the anti-hemorrhagic vitamin. As a matter of fact, these data do not furnish a sound basis for such finer reinterpretation. A variation in the results obtained from only one or two birds would have been sufficient to obliterate

the slight trends emphasized by Scott and Cook.

We have shown (Almquist, 1936) that prolonged clotting time and hemorrhage may be prevented in birds fed sardine meal diets by as little as one part of an anti-hemorrhagic vitamin concentrate in two million parts of diet. Studies of birds on such diets have also revealed that anemia does not occur when the minimum anti-hemorrhagic vitamin requirements are met and also does not exist in birds with prolonged blood clotting time when these birds are not afflicted by hemorrhage (Almquist and Stokstad, 1937). It is evident, therefore, that the amines in sardine meal could not be responsible for the anemia reported by Scott and Cook.

The red cell and white cell counts and hemoglobin levels reported in this paper fall within the limits for normal birds as reported by Cook and Dearstyne (1934) and Biely and Palmer (1935). These workers have reported extreme variability of red cell and particularly of white cell counts between individual, normal birds, a finding with which our data agree. For example, the ranges of variation in white cell count ($\times 10^3$) observed on the diets already described were, A, 8.7-34.0; B, 7.7-32.0; C, 7.0-27.0; D, 6.3-42.0; 4A, 4.7-24.3; 3C, 4.3-32.3; 2A, 7.0-28.0; modified, 14.0-32.0. Cook and Dearstyne (1934) reported a range of 3-47, Biely and Palmer (1935) a range of 18-49. These ranges may be regarded as very similar throughout. The average leucocyte counts reported by Scott and Cook (1936) for all diets, even those free of the "intoxication factor," are near or above the upper limits of the normal range. This suggests that some unrecognized factor may have contributed to their results.

The extreme variability in blood cell counts necessitates statistical treatment of such data in order to ascertain whether or not differences in the average blood composition of groups of chickens can be regarded as truly significant.

SUMMARY

1. Chicks raised on practical diets containing a standard feeding grade of sardine meal gave no evidence of prolonged blood clotting time, leucocytosis, hemorrhage, or anemia alleged to be caused by the "intoxication factor" in sardine meal. Blood characteristics of such chicks were the same as those raised on diets free of the "intoxication factor," or on diets containing added nitrogenous bases.

2. A comparison of casein and sardine meal in experimental diets produced no evidence of leucocytosis or anemia. Prolonged blood clotting time and hemorrhage were found only when the diet was deficient in the anti-hemorrhagic vitamin.

ACKNOWLEDGMENT

The authors wish to acknowledge the cooperation of C. A. Gunns in making the blood cells counts, and of E. Mecchi in the chemical analyses. Valuable assistance was also furnished by the Works Progress Administration.

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Book Reviews

Benjamin, E. W. and H. C. Pierce; *Marketing Poultry Products*, third edition. New York: John Wiley & Sons, Inc. London: Chapman & Hall, Limited, 1937. 401 pages, 212 figures. Price $4.00.

Although appearing under the old and well known title, the third edition of "Marketing Poultry Products" is, practically speaking, a new book. It is as up-to-date as a book that deals with so inconstant an industry can be. The excellent standards of thorough-going discussion and complete coverage of the new along with the well established practices in the handling of poultry and eggs that were set by the earlier editions have been carried forward in the present volume. Another reviewer has already cited the disappointment with which the individual who delights in destructive criticism must review this book.

The authors need little introduction to students of the marketing of poultry products. Each has a background rich in the experience of commercial responsibility built on a foundation of teaching and research training.

The authors have drawn freely upon the experience of others when necessary to supplement their own immediate fields of knowledge. Their expressed objective is to make "the study of marketing problems in the egg and poultry industry of greater interest to producers, students, research workers, operators and dealers." In doing this, they have prepared a book that serves well as a text, a reference and a manual of good practice. An unusually comprehensive bibliography and index are included as a fitting ending to a book that covers the field of marketing poultry products from the "A. B. C.—live poultry grades" to the "yields of dried yolk." The mechanical structure of the book will be recognized by those familiar with the earlier editions, but the reader will find it the most complete story of the industry as it stands today.

ALFRED VAN WAGENEN
Cornell University, Ithaca, N.Y.

Thompson, Willard C.; *Egg Farming*, New York: Orange Judd Publishing Co., Inc., 1936. 335 pages, 70 illustrations. Price $2.00.

The nature of this book is well indicated on the title page when it says that it is "a practical reliable manual upon producing eggs and poultry for market as a profitable business enterprise." It is intended as a handbook for the practical poultry farm operator who is engaged in egg farming as a business, although the beginning student can also use it to advantage.

The book deals specifically with the breeds to use, poultry laying flock improvement, maintaining health and productivity, chick production, economical and efficient housing, practical feeding programs and the handling, candling, grading, packaging and marketing of eggs. Thus, it considers all the steps or operations upon which the farmer might need advice or instruction.

In covering these subjects the principles involved are presented. In the application of these principles to practice, however, the author gives very specific recommendations which are based on his more than twenty years of experience in poultry education and research and his contact with poultry keeping, particularly table egg farming.

G. F. HEUSER

The Effect of Supplementary Heat on Egg Production, Feed Consumption, Amount of Litter Required, and Net Flock Income

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(Received for publication, March 5, 1937)

VERY little scientific investigation of the effect of supplementary heat, during the colder months, in the laying house, has been reported. Numerous articles in popular poultry magazines and farm papers have seemed to indicate the desirability of some supplementary heat during the colder months, at least in the northern states. However, very little conclusive evidence has been presented.

Among the papers presented at the Twenty-Eighth Annual Meeting of the Poultry Science Association, August 4-7, 1936, at the Virginia Polytechnic Institute, were papers by F. L. Fairbanks, Cornell University, and J. H. Bruckner, New York State Conservation Department, stationed at Cornell University.

These studies seemed to throw some doubt on the desirability of heat, particularly if the temperature could be kept above freezing without supplementary heat.

The investigation reported herein was begun in September, 1935, and is expected to extend through the next several years. This report covers only the results of the first year's work.

For this experiment eight 24 by 24 feet pens in two, two-third span roof, houses built on identical plans and standing within 100 yards or so of each other were used.

These houses were insulated and artificially ventilated. The ventilation systems used in the two houses were not the same and for this reason they were entirely sealed up during the period of this experiment and all ventilation was secured through operation of the sliding Cel-O-Glass frames in the front of each pen, which were identical in all pens.

The heated pens were equipped with six 1¼-inch pipes running along the north wall, back of the dropping boards, in each pen. These connected with a hot water boiler equipped with an efficient thermostatic control.

Artificial heat was supplied in the four heated pens from November 26, 1935, to March 24, 1936, or a period of 120 days. The inside pen temperatures, in the heated pens, averaged from 38 to 45 degrees. Every effort was made to keep the temperature at as close to 40 degrees as possible, having the thermometer 12 inches from the floor, on an end wall in each pen.

The average temperature of the unheated pens was 29.68 degrees for January, 33.79 degrees for February, and 48.67 degrees for March. The lowest temperature occurred on January 24, being 12 degrees above zero inside one of the pens in the unheated section.

House 2 in this experiment was not supplied with artificial heat, while house 3 was

TABLE 1.—*House 2. Egg production, income, expenses and net returns per bird. Pen 1 (no heat). Number birds at start, 159. Age at start, eight months. Breed: New Hampshires*

Month	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Net return per bird
1935									
Sept.	3.6	12.1¢	1.49	4.1¢	1.4	2.6¢			5.4¢
Oct.	12.27	45.1¢	4.36	12.5¢	3.68	7.4¢			25.2¢
Nov.	13.17	43.4¢	4.99	14.5¢	3.8	6.8¢	3.3¢	.9¢	17.9¢
Dec.	11.83	34.5¢	3.44	10.3¢	4.59	8.3¢	2.9¢	.96¢	12.0¢
1936									
Jan.	6.78	15.1¢	3.02	9.4¢	4.47	8.0¢	1.0¢	1.0¢	−4.3¢
Feb.	5.54	14.6¢	3.26	10.1¢	4.07	7.0¢	1.1¢	1.0¢	−4.6¢
March	12.08	24.9¢	4.25	13.2¢	4.04	7.3¢			4.4¢
April	17.22	34.0¢	4.42	13.7¢	4.1	7.4¢	1.7¢	1.2¢	10.0¢
May	17.00	39.9¢	3.83	11.5¢	4.31	7.3¢	1.2¢	1.2¢	18.7¢
June	14.56	34.4¢	3.97	11.5¢	3.97	7.1¢			15.8¢
July	11.35	35.1¢	3.62	12.7¢	3.77	8.7¢			13.7¢
Aug.	11.86	34.4¢	2.97	11.0¢	3.14	7.8¢			15.6¢
Sept.	5.4	16.1¢	.46	1.7¢	1.26	3.1¢			11.3¢
Totals for year	142.66	$3.84	44.1	$1.36	46.6	88.8¢	11.2¢	6.3¢	$1.41

the heated house. In the four 24 by 24 feet pens in house 2 we placed one group of 159 New Hampshire pullets and three groups of White Leghorn pullets of 214 birds each. In house 3 there were placed two groups of White Leghorns of 214 birds in each pen and two groups of New Hampshires, one group containing 159 pullets and the other 160 pullets.

The New Hampshire pullets were eight months of age at the start of the experiment and had been laying on range and in other pens for three and a half months and the moving incidental to the arrangement of the birds in pens for this experiment sent the New Hampshires into a decided molt from which they gradually came back.

The White Leghorns were six months of

TABLE 2.—*House 2. Egg production, income, expenses and net returns per bird. Pen 2 (no heat). Number birds at start, 214. Age at start, six months. Breed: White Leghorn*

Month	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Net return per bird
1935									
Sept.	.18	.5¢							.5¢
Oct.	5.52	10.5¢	2.19	6.3¢	4.06	8.0¢			−3.8¢
Nov.	9.68	20.7¢	2.92	8.5¢	3.36	6.0¢			6.2¢
Dec.	8.66	21.9¢	2.31	6.9¢	3.99	7.2¢	2.2¢	.7¢	4.9¢
1936									
Jan.	12.09	28.3¢	3.64	11.3¢	3.7	6.7¢	.8¢	.7¢	8.8¢
Feb.	14.15	38.6¢	4.53	14.0¢	3.7	6.7¢	.8¢	.8¢	16.3¢
March	19.41	39.4¢	4.5	14.0¢	4.23	7.6¢	.9¢	.8¢	16.1¢
April	21.06	40.5¢	3.39	10.5¢	4.33	7.8¢			22.2¢
May	19.83	39.5¢	3.83	11.5¢	4.5	7.6¢	1.0¢	.9¢	18.5¢
June	18.64	39.9¢	4.16	12.0¢	4.3	7.7¢			20.2¢
July	14.82	36.9¢	4.47	15.6¢	3.37	7.7¢			13.6¢
Aug.	13.57	37.6¢	3.89	14.4¢	3.18	8.0¢			15.2¢
Sept.	6.04	17.4¢	1.72	6.2¢	1.27	3.2¢			8.0¢
Totals for year	163.63	$3.72	41.55	$1.31	43.99	84.0¢	5.7¢	3.9¢	$1.47

TABLE 3.—*House 2. Egg production, income, expenses and net returns per bird. Pen 3 (no heat). Number birds at start, 214. Age at start, six months. Breed: White Leghorn*

Month	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Net return per bird
1935									
Sept.	.23	.7¢							.7¢
Oct.	6.03	29.2¢	2.16	6.2¢	4.19	8.4¢			14.6¢
Nov.	7.99	21.2¢	2.88	8.4¢	3.24	5.8¢			6.6¢
Dec.	7.78	19.7¢	2.69	8.1¢	3.81	6.8¢	2.6¢	.7¢	1.5¢
1936									
Jan.	12.4	29.0¢	3.94	12.2¢	3.64	6.6¢	1.4¢		8.8¢
Feb.	14.23	38.8¢	3.63	11.3¢	3.35	6.0¢	.8¢	.7¢	20.0¢
March	18.66	37.8¢	4.15	13.0¢	3.73	6.7¢	.8¢	.75¢	16.5¢
April	20.46	39.4¢	3.68	11.4¢	3.8	6.8¢			21.2¢
May	20.4	40.6¢	3.53	10.6¢	4.2	7.1¢	.9¢	.9¢	21.1¢
June	17.37	37.9¢	3.71	10.7¢	4.05	7.3¢			19.9¢
July	14.25	35.5¢	4.03	14.1¢	3.32	7.6¢			13.8¢
Aug.	14.22	39.4¢	2.79	10.3¢	3.21	8.0¢			21.1¢
Sept.	5.92	17.0¢	1.49	5.3¢	1.3	3.3¢			8.4¢
Totals for year	159.94	$3.86	38.68	$1.22	41.84	80.4¢	6.5¢	3.05¢	$1.74

age at the beginning of the experiment and had already begun to lay on the range when they were brought in. For best results the birds should have been housed a month earlier, or nearly so, but it was impossible to get the pens ready before September.

It should be noted that all the stock used in this experiment was very ordinary stock such as would be likely to be used by the average northeastern poultryman. It was all purchased from hatcheries, did not include any pedigreed stock of any kind, and was bought with only one specification, that it be free from pullorum. Both the New Hampshires and the Leghorns each came from one particular flock mating.

TABLE 4.—*House 2. Egg production, income, expenses and net returns per bird. Pen 4 (no heat). Number birds at start, 214. Age at start, six months. Breed: White Leghorns*

Month	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Net return per bird
1935									
Sept.	.26	.7¢							.7¢
Oct.	6.74	18.7¢	2.02	6.0¢	4.28	8.6¢			4.1¢
Nov.	11.16	29.5¢	2.93	8.5¢	3.3	6.0¢			15.0¢
Dec.	10.95	27.7¢	2.74	8.2¢	3.87	7.0¢	2.1¢	.7¢	9.7¢
1936									
Jan.	13.02	30.4¢	4.17	12.9¢	3.69	6.6¢	.8¢	.7¢	9.4¢
Feb.	15.23	41.5¢	3.95	12.2¢	3.49	6.3¢	.8¢	.8¢	21.4¢
March	19.36	39.3¢	4.52	14.0¢	3.88	10.0¢	.9¢	.8¢	13.6¢
April	19.52	37.6¢	3.4	10.5¢	3.97	7.1¢			20.0¢
May	18.53	36.9¢	3.08	9.2¢	4.09	6.9¢	.9¢	.9¢	19.0¢
June	16.05	34.3¢	3.35	9.7¢	3.77	6.8¢			17.8¢
July	14.57	36.3¢	2.99	10.5¢	3.22	7.4¢			18.4¢
Aug.	12.43	34.5¢	2.37	8.8¢	3.26	8.1¢			17.6¢
Sept.	4.77	14.0¢	1.54	5.5¢	1.28	3.2¢			5.3¢
Totals for year	162.59	$3.81	37.06	$1.16	42.10	84.0¢	5.5¢	3.9¢	$1.72

TABLE 5.—*House 3. Egg production, income, expenses and net returns per bird. Pen 1 (heated). Number of birds at start, 214. Age at start, six months. Breed: White Leghorns*

Month	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Pounds coal used per bird	Cost of coal per bird	Net return per bird
1935											
Sept.	.06	.2¢									.2¢
Oct.	5.10	14.1¢	1.77	5.1¢	3.5¢	.7¢					8.3¢
Nov.	10.67	28.2¢	2.98	8.6¢	3.29	5.9¢			1.0	.6¢	
Dec.	13.07	33.1¢	2.5	7.5¢	3.78	6.8¢	1.4¢	.7¢	6.29	3.9¢	12.8¢
1936											
Jan.	13.92	32.6¢	2.85	8.8¢	3.16	5.7¢	.2¢	.7¢	6.61	4.1¢	13.1¢
Feb.	14.27	38.9¢	3.93	12.2¢	3.03	5.4¢			6.61	4.1¢	17.2¢
March	17.38	35.3¢	3.84	11.9¢	3.26	5.7¢			5.81	3.6¢	14.1¢
April	13.65	26.3¢	2.62	8.1¢	3.79	6.8¢	.9¢	.8¢			9.2¢
May	12.7	25.3¢	2.93	8.8¢	3.72	6.3¢	.9¢	.9¢			8.4¢
June	13.88	29.7¢	2.67	7.7¢	3.79	6.8¢					15.2¢
July	11.7	29.2¢	1.61	5.6¢	3.24	7.4¢					16.2¢
Aug.	10.68	29.6¢	1.75	6.5¢	3.37	8.4¢					14.7¢
Sept.	5.35	15.6¢	.58	2.1¢	1.33	3.3¢					10.2¢
Totals for year	142.43	$3.38	30.03	93.0¢	39.26	69.0¢	3.4¢	3.1¢	26.32	16.3¢	$1.53

The pullets were carefully selected and were put into the pens so as to give as absolutely uniform birds in each pen as possible. All the birds had been reared on the same rations and under the same program on the same range.

Tables 1 to 10 inclusive, show the egg production per bird in each pen, the actual income per bird derived from the sale of these eggs, the pounds of mash fed per bird and the cost of mash, the pounds of grain per bird and the cost of that grain, the litter cost and the labor cost for changing the litter, and finally the net return per bird

TABLE 6.—*House 3. Egg production, income, expenses and net returns per bird. Pen 2 (heated). Number of birds at start, 214. Age at start, six months. Breed: White Leghorn*

Month	Eggs per bird	Actual income per bird	pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Pounds coal used per bird	Cost of coal per bird	Net return per bird
1935											
Sept.	.15	.4¢									.4¢
Oct.	5.35	14.7¢	1.52	4.3¢	3.38	6.8¢					3.6¢
Nov.	10.25	27.1¢	2.96	8.6¢	3.23	5.8¢			1.0	.6¢	12.1¢
Dec.	12.57	31.8¢	2.17	6.5¢	3.65	6.6¢	1.3¢	.7¢	6.12	3.8¢	12.9¢
1936											
Jan.	11.44	26.8¢	2.97	9.2¢	3.16	5.7¢	.2¢	.7¢	6.29	3.9¢	7.1¢
Feb.	11.47	31.3¢	2.75	8.5¢	3.1	5.6¢			5.97	3.7¢	13.5¢
March	13.63	27.7¢	3.48	9.2¢	3.21	5.7¢			5.16	3.2¢	9.6¢
April	14.03	27.0¢	2.46	7.6¢	3.55	6.4¢	.8¢	.8¢			11.4¢
May	12.92	25.7¢	2.73	8.2¢	3.55	6.0¢	.8¢	.8¢			9.9¢
June	10.43	22.3¢	2.44	7.1¢	3.53	6.3¢					8.9¢
July	7.86	19.6¢	1.33	4.7¢	3.14	7.2¢					7.7¢
Aug.	8.18	22.7¢	1.43	5.3¢	2.98	7.4¢					10.0¢
Sept.	4.42	12.7¢	.54	1.9¢	1.18	3.0¢					7.8¢
Totals for year	122.7	$2.90	26.78	81.0¢	37.66	73.0¢	3.1¢	3.0¢	24.54	15.2¢	$1.15

TABLE 7.—*House 3. Egg production, income, expenses and net returns per bird. Pen 3 (heated). Number of birds at start, 159. Age at start, eight months. Breed: New Hampshires*

Month	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Pounds coal used per bird	Cost of coal per bird	Net return per bird
1935											
Sept.	9.78	32.8¢	1.45	4.0¢	1.69	3.2¢					25.6¢
Oct.	12.57	45.9¢	4.11	11.5¢	3.25	6.5¢					27.9¢
Nov.	10.83	35.7¢	3.53	10.2¢	3.56	6.4¢	2.8¢	.9¢	1.29	.8¢	14.6¢
Dec.	9.61	28.1¢	3.66	11.0¢	4.46	8.0¢			8.71	5.2¢	3.9¢
1936											
Jan.	10.28	24.0¢	3.34	10.4¢	4.32	7.8¢			8.87	5.4¢	.4¢
Feb.	7.34	19.7¢	2.94	9.1¢	3.88	7.0¢	1.1¢	1.0¢	8.71	5.2¢	−3.7¢
March	8.09	25.5¢	3.07	10.3¢	4.11	7.8¢			7.42	4.7¢	2.7¢
April	12.01	35.0¢	3.23	10.0¢	4.22	7.6¢	1.2¢	1.0¢			15.2¢
May	12.62	35.3¢	3.39	10.2¢	3.71	6.3¢	1.2¢	1.2¢			16.4¢
June	11.8	35.1¢	3.23	9.4¢	3.5	6.3¢					19.4¢
July	9.61	36.8¢	2.03	7.1¢	3.63	8.3¢					21.4¢
Aug.	9.26	37.2¢	3.04	11.2¢	3.4	8.5¢					17.5¢
Sept.	4.19	17.7¢	.88	3.2¢	1.39	3.5¢					11.0¢
Totals for year	127.99	$4.09	37.90	$1.18	45.12	87.0¢	6.3¢	4.1¢	35.0	21.3¢	$1.72

average for each pen.

The income was found, from each pen, not by simply crediting the pen with so many dozens of eggs at the average market price but by computing the actual value of the eggs laid on the basis of the sizes and the net returns actually received for each particular grade in the shipment in which the particular eggs were included. Shipments were made to New York City twice a week and the eggs were sold at two cents premium over the regular price per dozen for eggs of that grade.

TABLE 8.—*House 3. Egg production, income, expenses and net returns per bird. Pen 4 (heated). Number of birds at start, 160. Age at start, eight months. Breed: New Hampshires*

Month	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Pounds coal used per bird	Cost of coal per bird	Net-return per bird
1935											
Sept.	12.35	41.4¢	1.39	3.8¢	1.42	2.7¢					34.9¢
Oct.	12.42	45.4¢	4.18	11.7¢	3.3	6.6¢					27.1¢
Nov.	11.22	36.9¢	3.6	10.4¢	3.62	6.5¢	2.9¢	.9¢	1.29	.8¢	15.4¢
Dec.	9.57	27.9¢	4.43	13.2¢	4.56	8.2¢			8.71	5.4¢	1.1¢
1936											
Jan.	11.25	26.2¢	3.69	11.4¢	4.32	7.8¢			8.87	5.5¢	1.5¢
Feb.	8.91	23.8¢	3.74	11.6¢	4.12	7.4¢	1.1¢	1.0¢	8.71	5.4¢	−2.7¢
March	13.88	28.7¢	4.57	11.4¢	4.06	7.8¢			7.42	4.6¢	4.9¢
April	14.35	28.3¢	4.07	12.6¢	4.24	7.6¢	1.2¢	1.0¢			5.9¢
May	12.91	26.5¢	5.02	15.1¢	4.05	6.9¢	1.2¢	1.0¢			2.3¢
June	11.62	27.5¢	3.99	11.6¢	3.38	6.1¢					9.8¢
July	8.86	27.4¢	2.77	9.7¢	3.31	7.6¢					10.1¢
Aug.	8.28	24.0¢	3.66	13.5¢	3.0	7.5¢					3.0¢
Sept.	3.83	11.4¢	.83	3.0¢	1.22	3.0¢					5.4¢
Totals for year	139.45	$3.75	45.94	$1.39	44.6	86.0¢	6.4¢	3.9¢	35.0	21.7¢	$1.19

TABLE 9.—*House 2 (no heat). Summary of egg production, income, expenses and net returns per bird*

Period	Eggs per bird	Actual income per bird	Pounds mash fed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Net return per bird
New Hampshires									
Sept. 1935 Through Sept. 1936	142.66	$3.84	44.1	$1.36	46.6	88.8¢	11.2¢	6.3¢	$1.41
White Leghorns									
Sept. 1935 Through Sept. 1936	162.05	$3.80	39.09	$1.23	42.64	82.8¢	5.9¢	3.6¢	$1.64

DISCUSSION

The New Hampshires in the unheated house averaged nearly nine eggs per bird above those in the heated pens. Considering the fact that only one pen of New Hampshires was available for the unheated house, this result may not give a true indication of the effect of heat but considering the fact that the egg production of both the heated pens was so far below the birds in the unheated pens, and considering also that these birds were all of very uniform appearance when they were put into the pen, this result may really be an accurate index. Later years will give us additional data.

The New Hampshires in the heated pens, even though but very little heat was supplied, were quite evidently less active than the birds in the unheated pens, and much of the time did not exhibit keenness of appetite, alertness, and other indications which we associate with top-notch condition and egg production, to the same extent shown by the birds in the unheated pens.

While it might be expected that the heavier breeds, such as New Hampshires, which are known to be more resistant to cold weather, would not benefit as much by the addition of heat as the White Leghorns, it was rather surprising to note that the White Leghorn pens also averaged nearly 30 eggs per bird higher production in the unheated pens than in the heated ones. The heated Leghorns laid well during cold weather but they all slipped in production

TABLE 10.—*House 3 (heated). Summary of egg production, income, expenses and net returns per bird*

Period	Eggs per bird	Actual income per bird	Pounds mash feed per bird	Cost of mash per bird	Pounds grain fed per bird	Cost of grain per bird	Litter cost	Labor cost for litter changes	Pounds coal used per bird	Cost of coal per bird	Net return per bird
New Hampshires											
Sept. 1935 Through Sept. 1936	133.72	$3.92	41.92	$1.28	44.86	86.5¢	6.35¢	4.0¢	35.0	21.5¢	$1.46
White Leghorns											
Sept. 1935 Through Sept. 1936	132.56	$3.14	28.40	$.87	38.46	71.0¢	3.25¢	3.05¢	25.43	15.75¢	$1.34

much earlier in the summer than the birds that had not received supplementary heat during the previous winter.

It is possible that here again we have an illustration of the effect of forcing birds to lay at an unnatural season and that, as usual under similar conditions, the birds that lay the greater portion of their eggs during the more natural laying season will make the highest annual production, other factors being the same.

CONCLUSIONS

So far as conclusions may be drawn from the first year of this experiment it seems obvious that annual egg production both in New Hampshires and in White Leghorns is lowered by the use of supplementary artificial heat in an insulated laying house. Whether this would be equally true in an uninsulated laying house may be questioned but since a house may be insulated at a lower cost, over a period of years, than it may be satisfactorily heated, it would seem obvious that for commercial practice in the northeastern states we would be on safer ground in recommending insulation rather than artificial heat.

The feed consumption per bird was reduced about 5 pounds per bird for the New Hampshires in the heated house and about 15 pounds per bird in the case of the Leghorns.

Litter cost was nearly cut in two by the use of heat and, of course, labor of changing litter was also reduced.

The coal consumption per bird was hardly significant in the case of the New Hampshires because the total capacity of the pen was not utilized and this made the coal consumption per bird run somewhat higher than would have been the case, although the difference was not great. With the heating system used in this experiment it apparently was necessary to consume from 25 to 35 pounds of coal per bird per year to secure an average temperature of 40 degrees in the heated pens.

The net return per bird is not significantly different in the heated and unheated pens with New Hampshires but does seem to be significantly different, in favor of the unheated pens, with the White Leghorns.

Since this is a preliminary, and not a final report, detailed conclusions may be made only tentatively and complete comparisons will be deferred until the presentation of the final report after several years' work.

The Influence of Rations on the Annual Mean Egg Weight of Pullets[1]

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INTRODUCTION

A NUMBER of investigators have reported that the ration of a hen will influence the weight of eggs she lays. While an extensive review of the literature relating to this subject will not be presented at this time, reports of positive results have been made by Parkhurst (1924, 1926, 1927, and 1928), Kistler, Charles, and Knandel (1926), and Kempster (1930). Graham (1932) reports *no* influence of protein on mean egg size during February to July in the case of 913 Barred Rock pullets. In some cases the previous evidence is based on weights of only a portion of the total egg production, and in other cases the rations were fed in such a way that a reasonably accurate estimate of the ratio of the consumption of grains to protein concentrates could not be determined at any particular time for individual hens. It seemed desirable, therefore, to obtain more information on the subject with the use of the "all-mash" method of feeding and by weighing each egg produced.

EXPERIMENTAL METHOD

When this project was initiated it was assumed that the factors which might conceivably influence egg weight could be grouped into at least two categories, namely, breeding and environment. The breeding was perhaps partially controlled by selecting full and half sisters for the experimental pens. The general environment although not controlled was reasonably uniform for each pen. The principal variables in the environment were assumed to be the rations.

In order to test the influence of varying percentages of different kinds of protein concentrates on egg size, eight pens of 30 Single Comb White Leghorn pullets were selected October 1, 1930. The basal mash, which was fed to all groups, consisted of one part by weight of ground yellow corn, two parts of ground wheat, and one part of ground oats. To this mixture was added 3 percent of steamed bone meal. The pullets in the eight different pens received the following protein concentrates in their mash:

Pen 1. Fifteen (15) percent dried buttermilk.
Pen 2. Ten (10) percent dried buttermilk.
Pen 3. Five (5) percent dried buttermilk.
Pen 4. Ten (10) percent meat and bone meal.[3]
Pen 5. Five (5) percent buttermilk and five (5) percent meat and bone meal.[3]
Pen 6. Nine (9) percent buttermilk and five (5) percent meat and bone meal.[3]
Pen 7. Five (5) percent dried buttermilk and eight (8) percent meat and bone meal.[3]
Pen 8. Five (5) percent meat and bone meal.[3]

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[2] The suggestions of Professors G. W. Snedecor and A. E. Brandt of the Statistical Laboratory are gratefully acknowledged. Miss Gertrude M. Cox of the Statistical Laboratory supervised computations.

[3] Fifty (50) percent protein.

Cod liver oil at the rate of 1 percent of the total ration was added during the period from November 15 to March 1, after which free range on grass was allowed when the weather permitted. Oyster shell and gravel were fed free choice to all pens. The method of management was the same for all pens and they were housed consecutively in a continuous type of laying house. The eggs produced were marked individually, gathered daily and stored in a basement until the following morning when they were removed for weighing. However, attention should be called to the fact that the indicator dial of the balance used to weigh the eggs was graduated only to two gram intervals. This necessitated visual interpolation which was considered to be without bias and reasonably accurate to one gram. The pullets were weighed individually each month.

TABLE 1.—*Monthly and annual mean egg weight by pens*

Pen No.		Oct.	Nov.	Dec.	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug	Sept.	Annual Mean
1	15% D.B.*	42.8	45.5	44.2	48.9	49.6	50.1	50.4	50.7	50.3	49.0	50.8	50.6	48.6
2	10% D.B.	44.2	46.6	47.0	49.5	49.0	51.3	50.7	50.5	49.3	49.0	50.5	42.2	49.2
3	5% D.B.	43.7	45.9	47.9	50.1	49.8	52.3	52.6	52.9	51.6	48.7	52.1	53.2	50.1
4	10% M. & B.M.†	41.7	44.0	44.8	47.6	49.1	50.6	50.5	50.9	49.5	48.9	50.2	52.2	48.4
5	5% D.B. 5% M. & B.M.	43.5	46.1	47.5	50.5	51.5	52.3	52.2	51.9	50.2	49.8	51.2	54.7	49.8
6	9% D.B. 5% M. & B.M.	43.8	46.4	46.9	50.6	51.4	51.4	52.2	51.8	50.7	49.7	49.8	53.6	49.5
7	5% D.B. 8% M. & B.M.	43.0	45.0	46.3	49.6	49.6	51.4	51.3	50.9	49.1	49.1	49.9	50.5	48.6
8	5% M. & B.M.	42.5	44.7	46.7	48.7	49.5	51.7	51.9	51.9	50.8	50.3	52.1	52.7	49.7
	Mean	43.2	45.6	46.6	49.4	49.9	51.4	51.5	51.5	50.2	49.3	50.9	52.4	49.3

* D.B. = Dried Buttermilk.
† M. & B.M. = Meat and Bone Meal.

ANALYSIS OF RESULTS

General Significance

The mean egg weights for each pen by months and for the year are shown in Table 1.

It is evident from the table that the average egg weight of all pens is increasing from month to month until the month of April. A decline in egg weight occurs during the summer months but the maximum average egg weight of all pens combined is not attained until September. This situation suggests the question of how the influence of rations on egg weight should be measured. It is conceivable that the influence of rations might be measured in several ways. One method might be to determine the average weight of all eggs laid in each pen during the year. This method is open to the criticism that individual hens which laid the larger number of eggs would have an undue influence on the mean. It is also certain that different hens lay the larger share of their annual production during different periods of the year. Weights of eggs thus produced could, therefore, exert an unbalanced influence on the mean.

In the final tabulation of results the pro-

TABLE 2.—*Analysis of variance of mean egg weight of pullets, year 1930–1931*

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Between means of months*	11	13,277.62	1,207.06†
Between means of pen*	7	719.57	102.80†
Interaction*	77	700.95	9.10
Experimental error	1,780	14,384.80	8.08
Total	1,875	34,884.92	

* Disproportionate Sub-class Numbers. (7)
† Indicating P .01.

duction of all pullets which laid during each month was used to calculate the mean for the month. In calculating the monthly mean for each pen, the influence of each individual hen in the pen is reduced to unity by calculating the mean weight of all the eggs she laid. This method is subject to the criticism that a pullet which died and did not lay throughout the 12 month period would still exert an influence on the annual mean. It is likewise true that various other pullets may not have been in production during that particular time. A more ideal method might be to consider only those pullets which survived the 12 month period. In this case, however, the numbers for some of the months would have been materially reduced. In one pen only one pullet laid during each of the 12 months and there were no pens in which more than eight pullets produced continuously for the entire 12 month period.

For purposes of this study the annual mean for each pen is the average of the 12 monthly means.

The test for general significance of the means between pens and between months is indicated in Table 2.

The Influence of Quantity of Protein

While there is considerable variation in the amount of the several protein supplements added the differences between the mean annual egg weights of the pens are relatively small. General significance is indicated by statistical analysis, but the differences are not consistent in many cases with expectations based on the work of others. For instance, in the case of the milk supplements the annual mean egg weight increases with the decrease in percentage of milk in the ration.

An examination of the other supplement groups, that is, the group of pens which received combinations of milk, meat and bone meal (pens 5, 6 and 7), and the meat and bone meal supplemented pens (4 and 8) reveals that egg weight declines with the increased amount of supplement. Since the increase in the amount of supplement obviously results in an increased percentage of protein the egg weights of the several pens are tabulated in relation to the estimated percentage of protein in the ration. The percentage of protein was calculated partly from known chemical analysis and partly from average analyses of the ingredi-

TABLE 3.—*Regression of mean annual egg weight on percent protein content of ration*

	Pen Numbers							
	3	8	2	5	1	4	6	7
Percent protein	12.32	12.90	13.41	13.96	14.40	14.52	14.75	14.88
Egg weight (Gms.)	50.1	49.7	49.2	49.8	48.6	48.4	49.5	48.6

ents as compiled by Henry and Morrison (1928). They are shown in Table 3.

The data are presented graphically in Figure 1 where egg weight is plotted against the percentage of protein in the several rations of the eight pens. The regression line fitted by the method of least squares indicates clearly that increased percentages of protein resulted in *decreased* egg weight because the trend is highly significant.

It will be noted, however, that the range in percentage of protein of the entire group is narrow. Likewise, the range in variation of egg weight is small. What would happen to weight if higher or lower percentages of protein were fed must be left to conjecture at this time. It is conceivable that the regression of egg weight on percentage of protein might not be linear if more extreme percentages of protein should be fed. In three cases out of four (pens 1, 4, and 7) the smaller annual mean egg size is significantly associated with the higher percentages of protein in the rations. In the case of pen 6 this is not true, but when pen 6 is grouped with pens 1, 4, and 7, the mean of this group still exhibits a highly significant difference from the other four pens.

The annual mean egg size of the pullets in pens 3 and 8 (the low protein pens) is significantly greater than the mean of the other six pens.

In this particular experiment there is no question but what large egg size was clearly associated with the lower concentrations of protein supplement in the ration. This holds true in the case of milk, meat and bone meal, and combinations of the two.

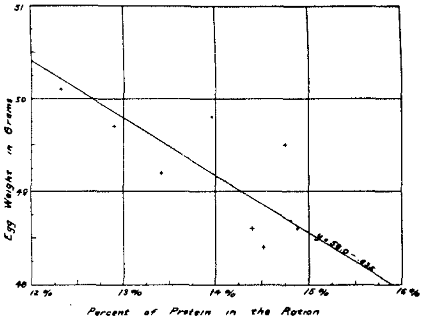

FIG. 1

Possible Contributing Factors

The discovery that annual mean egg weight was less as the percentages of protein in the rations increased from pen to pen was contrary to expectations based on reports of other stations. It seemed desirable, therefore, to extend the statistical analysis of the data in an attempt to find reasons for such apparently divergent results.

Before presenting the statistical treatment it will probably be helpful to consider some of the hypotheses which motivated the work.

One hypothesis which seemed worth investigating was that the rations containing the greater amounts of protein might have resulted in a more rapid rate of production. A rapid rate of laying might tend to reduce the number of pauses[4] between eggs. It may be assumed that a pause between the laying of eggs would result in larger average egg size for Atwood (1917) and Jull (1924) have shown that the first egg of a clutch[5]

[4] A pause is defined herein as one or more days of non-production.

[5] A clutch is defined herein as a group of eggs which have been laid on consecutive days.

is usually larger than the second egg and the last egg of a clutch is usually the smallest of that particular clutch.

Another possible inference which might explain the egg weight depressing influence of increased percentages of protein is that a pullet which is still growing while laying at a rapid rate might be unable to assimilate food materials fast enough to permit both the production of eggs of normal size and a normal growth rate. If this were true there should be a positive relationship between rate of production and concentrations of protein.

The relationship of pauses to numbers of eggs is not as simple as it might appear upon first observation for a pullet can lay the same number of eggs in a number of different sequences. For example, she could lay 15 eggs consecutively in one month and then cease laying for 15 or 16 days and register only one pause during that time. The same pullet might lay in an entirely different manner another month or another pullet might, for example, lay every other day, pause 16 times and lay a total of 15 eggs. The possible variations are obviously numerous.

If variations in the percentages of protein concentrate in the rations influence the number of eggs laid in a month's time, and the number of pauses or the size of clutch, the decline in annual mean egg size with increased percentages of protein might be partially explained.

Another possible relationship which seemed worth investigating was that of the concentration of protein and the rate of increase of egg weight especially during the period when pullets are growing.

To summarize, the objectives of the additional statistical analyses of the data which will be presented were to determine the relationship between the following factors: (1) rate of production and percentages of protein, (2) the number of pauses or clutch numbers and egg weight, (3) rate of production, clutch number and egg weight, (4) kind of protein and rate of increase in egg weight, (5) the different kinds of protein and annual mean egg weight.

TABLE 4.—*Analysis of covariance*
Egg weight on clutches

Source of variation	Degrees of freedom	Sum of egg weight	r	Errors of Estimate		
				Sum of squares	Degrees of freedom	Mean square
Between pens	7	723				
Within-error	1,780	17,339	.19*	16,725	1,779	9.40
Total (Pen ± Error)	1,787	18,063	.21	17,291	1,786	
For testing significance of pen differences				506.87	7	80.98†

* Statistically significant.
† Highly significant.

Rate of Production in Relation to Concentration of Protein

Rate of production might be measured in more than one way but in this work rate of production was taken as the number of eggs laid in a month's time. This method is subject to the criticism that the number of days in a month varies but nevertheless the method is very commonly used.

Rate of production was not affected by

the concentrations of protein in the ration. This is shown by the value of the regression of numbers of eggs per month on the percentage of protein which was found to be —.22. It is not statistically significant. This fact suggested that the greater concentrations of protein in the rations did not cause a reduction in egg size by stimulating an increased rate of production.

The Analysis of Covariance of Egg Weight and Number of Pauses or Clutches

The influence of the number of clutches on egg weight within the pens is shown by the value of the correlation coefficient +.19. It is highly significant but it is not large enough to account for much of the variation between pens. This is shown by the fact that after the influence of clutch number is accounted for by adjusting the sum of squares in Table 4 the difference between the mean squares within pens and between pens is still highly significant.

The influence of clutch number on egg weight is also evident from the regression of egg weight on clutch number both within pens and on the mean of the pens. The value of the regression coefficient within pens is .27, which is highly significant. Between the means of pens the regression coefficient is much higher, .65. It is also highly significant. These findings are in agreement with the reports of Atwood (1917) and Jull (1924) which showed that egg weight tends to increase as the number of pauses between clutches of eggs is increased.

EGG WEIGHT IN RELATION TO RATE OF PRODUCTION AND NUMBER OF CLUTCHES

Rate and Egg Weight

It has been suggested previously that the negative trend of egg weight with the concentrations of protein in the ration might be the reseult of an increased rate of production as the percentages of protein in the rations increased. Although there was no significant trend of rate of laying with pro-

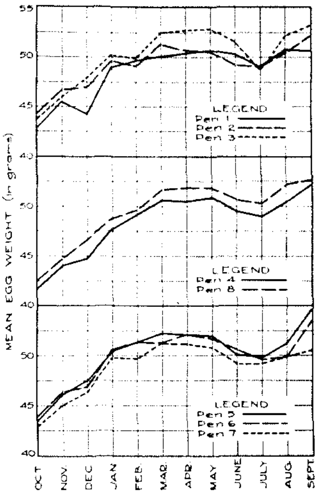

FIG. 2

tein, rate of laying might be associated with clutch number and egg size.

From a casual observation of Table 5 it is clear that the rate of production is fluctuating from month to month especially during the first four months. Egg weight, however, in all pens is steadily increasing during this same period as shown in Figure 2. This would indicate that for the first four months at least egg weight is certainly not adversely affected by numbers of eggs laid. It might appear that the decline in numbers of eggs per month during this period is exerting a positive influence on egg weight if it were not for the fluctuations

TABLE 5.—*Mean number of eggs per pullet*

Pen Number	Month						
	Oct.	Nov.	Dec.	Jan.	Feb.	March	April
1	10.2	8.2	6.7	14.8	17.3	20.4	18.9
2	9.1	3.0	9.0	15.4	15.8	19.2	20.0
3	9.2	6.2	9.0	14.1	15.5	19.5	19.7
4	7.8	1.5	4.5	11.4	16.0	17.4	17.1
5	8.8	9.7	8.7	16.0	15.9	19.6	18.3
6	5.0	9.0	11.0	13.1	18.1	20.3	19.9
7	9.0	6.0	8.0	15.1	15.4	19.7	19.1
8	5.1	9.5	7.0	11.2	14.5	16.9	17.9

in rate in contrast to the uniformly increasing curve of egg weight.

Any relationship between egg weight and rate of production during the first four months of production would be especially interesting for it is well known that most pullets are growing at this time.

Rate, Egg Weight and Number of Clutches for the Entire Year

While the previous rough comparison of the rate of production for the first 10 months with the curve of egg weight for that period is of some interest a more precise measure of the influence of rate of production on egg weight was obtained from the study of these variables throughout an entire year, by months. It was assumed that rate of production might be related to egg weight in more than one way. (1) A direct relationship between total *number of eggs laid in a month's time and* egg weight, and (2) an indirect relationship as a result of a reduction in the number of days of pauses between a series of days of laying. It is obvious, therefore, that the influence of number of eggs laid per month is dependent in part upon the number of pauses between days of production or in other words the number of clutches in which a given number of eggs may be laid. Therefore, it seemed desirable to include both the number of eggs per month and the number of clutches per month in an analysis of convariance.

The combined influence of numbers of eggs per month and number of pauses between clutches on egg weight is shown in Table 6. The multiple correlation coefficient R = .53 is fairly high and accounts for about 27 percent of the variance in egg weight. The pen differences in egg weight

TABLE 6.—*Analysis of covariance*
Number of eggs and clutches on egg weight

Source of variation	Degrees of freedom	Sum of squares of egg weight	R	Errors of Estimate		
				Adj. sum of squares of egg weight	Degrees of freedom	Mean square
Between pens	7	723				
Within-error	1,780	17,339	.53	12,539	1,778	7.52
Total (Pen+Error)	1,787	18,063	.51	13,425	1,785	
For testing significance of pen differences				885	7	126.51†

† Highly significant.

TABLE 7.—*Means of pens*

Pen No.	Mean No. eggs per month	Mean No. clutches per mo.	Observed mean weight	Estimated weight	Errors of estimate
1	14.32	5.51	48.61	49.01	−.40
2	17.53	6.09	49.21	49.96	−.75
3	14.35	6.82	50.27	49.12	1.14
4	14.99	5.99	48.40	49.24	−.84
5	14.81	5.75	49.75	49.17	.57
6	14.37	5.87	49.47	49.05	.41
7	15.03	5.92	48.63	49.24	−.61
8	15.45	6.37	49.68	49.39	.28

remain highly significant, however, after the influence of numbers of eggs and number of pauses between clutches is taken into account.

A table of errors of estimate based on the correlation between egg weight and number of eggs per month and number of clutches per month was calculated. The errors of estimate are shown in Table 7. It is clear from this table that there is considerable variation in egg weight between pens which is not accounted for by egg numbers and clutches. It may be attributed in large measure to the percentage of protein in the rations. This is more clearly shown in Table 8 where the errors of estimate are arrayed with the percentages of protein. It is evident from this table that egg weight tended to be greater than estimated in case of the lower concentrations of protein and lower in cases of the higher concentrations of protein.

No explanation is suggested for the exceptions in the cases of pens 2 and 6.

Additional evidence of the influence of rate of laying (numbers of eggs per month) on egg weight is obtained when the regression of egg weight on egg numbers per month is calculated. Within pens the value of this regression coefficient is .29. It is significant. This indicates that regardless of the ration the hens which lay the most eggs in a month's time tend to lay the heaviest eggs. This may be due in part to a tendency on the part of the hens which lay the greatest number of eggs to register the greatest number of pauses also.

The regression of egg weight on numbers of eggs per month between pens was negative but not significant. This would indicate that in spite of a general tendency on the part of the population of hens to lay heavier eggs as the number of eggs per month increased the depressing influence of the ration on egg weight was sufficient to overcome the tendency.

It seems, therefore, that increased percentages of protein reduce egg size independently of any effect on the rate of production.

Egg Weight in Relation to Rate of Increase in Egg Weight

Another fairly popular supposition which may offer an explanation of the depressing effect of high concentrations of protein on

TABLE 8.—*Deviations of errors of estimate of egg weight with percentages of protein*

Pen No.	3	8	2	5	1	4	6	7
% Protein	12.32	12.90	13.41	13.96	14.40	14.52	14.75	14.88
Error of Estimate	+1.15	+.28	−.76	+.58	−.40	−.84	+.41	−.62

annual mean egg weight is that the higher concentrations of protein stimulate pullets to lay eggs before they are "mature" in skeletal size but fail to stimulate a correspondingly rapid rate of increase of body size. In other words a ration which might stimulate rapid early production might not promote proportionately rapid growth of pulets over the same period. As a result the attainment of maximum body size and egg size might be delayed and therefore the annual mean egg weight might be reduced.

TABLE 9.—*Percentage rate* of increase in egg weight to April and percentage protein in rations*

	Pen numbers arranged according to increasing percent protein							
	3	8	2	5	1	4	6	7
Percentage rate of increase	2.64	2.87	1.96	2.60	2.31	2.72	2.50	2.52
Percentage protein	12.32	12.90	13.41	13.96	14.40	14.52	14.75	14.88

* Cite Brody.

A type of ration which might be expected to produce such an effect would be one in which there is a high concentration of protein with a relatively small amount of bone building materials especially calcium phosphate.

If this situation were operating to reduce egg size an examination of the rates of increase in egg size should yield differences. An examination of the data from this standpoint would have additional value if any particular ration proved superior.

Since skeletal measurements of the pullets were not taken it was not considered worth while to calculate the individual percentage growth rates of each pullet for purposes of analysing the variance.

An estimate of the effect of the percentages and kind of protein upon the rate of increase in egg weight may be obtained from an array of the mean "percentage rate"[6] of increase in egg weight with the percentages of protein in the rations as shown in Table 9. The rate of increase in egg weight may be estimated also from the apparent slope of the curves of egg weight in Figure 2, where the mean egg weight is plotted on a semi-logarithmic graph. It is well known that the use of this type of graph tends to convert curves of the exponential type into straight lines which facilitates visual impressions of rates of change.

There was no apparent relation between the mean rate of increase in egg weight and the percentages of protein in the ration. The most rapid rates of increase are apparent in pens 4 and 8. This is rather interesting since the hens in both these pens were fed meat and bone meal as a source of protein supplement. It was considered that all pens received sufficient phosphorus since they were all given three percent of bone meal. Pens 5, 6, and 7, however, received as much meat and bone meal as pen 8 but more protein in the form of milk which compared to meat and bone meal is relatively low in phosphorus. It will be noted also that the rate of increase in egg weight in pen 8 is greater than that in pen 4 which received twice as much meat and bone meal supplement. The eggs from pen 8 are consistently heavier during the period for which the rate of increase was calculated.

From these data it seems that the variations in rate of increase in egg weight be-

[6] Brody (1927).

tween pens are not related to the kind of protein concentrate in the rations.

SOURCE OF PROTEIN

Other workers have reported that protein concentrates from certain sources were superior to others in their influence on egg size. In order to test the relative egg weight stimulating properties of three kinds of protein supplements three groups of pens are compared. Group 1 consisted of pens 1, 2, and 3, which received approximately 15, 10, and 5 percent of dried skimmilk supplement respectively. Group 2 consisted of pens 4 and 8; they received approximately 10 and 5 per cent of meat and bone meal supplement respectively. Group 3 consisted of pens 5, 6, and 7. They received both milk and meat and bone meal supplements in varying quantities as indicated in the description of experimental methods. The analysis of variance between and within groups yielded the results shown in Table 10. It is evident that the difference in mean egg weight between the groups is not significant when compared to the difference between pens.

TABLE 10.—*Analysis of variance between groups*

Source of variation	Degrees of freedom	Sum of squares	Mean squares
Between pens	7	723.82	103.40
Between groups	2	29.30	14.65
Between pens of the same group	5	694.52	138.90

SUMMARY

The annual mean size of all eggs laid by Single Comb White Leghorn pullets on eight different all-mash rations varied from 48.4 to 50.1 grams. The rations fed were as follows: Basal: 50 parts of ground wheat, 25 parts of ground oats, 3 parts of steamed bone meal, and 1 part of salt. The protein concentrates added to the basal ration of the several pens were as follows:

Pen 1. Fifteen (15) percent dried buttermilk.
Pen 2. Ten (10) percent dried buttermilk.
Pen 3. Five (5) percent dried buttermilk.
Pen 4. Ten (10) per cent meat and bone meal.[7]
Pen 5. Five (5) percent buttermilk and five (5) percent meat and bone meal.[7]
Pen 6. Nine (9) percent buttermilk and five (5) percent meat and bone meal.[7]
Pen 7. Five (5) percent dried buttermilk and eight (8) percent meat and bone meal.[7]
Pen 8. Five (5) percent meat and bone meal.[7]

Cod liver oil at the rate of 1 percent of the total ration was added during the period from November 15 to March 1, after which free range on grass was allowed when the weather permitted.

The annual mean egg weight in grams produced by the eight pens indicated was as follows:

Pen 1. 48.6	Pen 5. 49.8
Pen 2. 49.2	Pen 6. 49.5
Pen 3. 50.1	Pen 7. 48.6
Pen 4. 48.4	Pen 8. 49.7

General significance between pens was indicated by statistical analysis. The pens receiving the higher concentrations of protein supplement laid the smallest eggs, the differences being highly significant.

Increased concentrations of protein in the ration reduced numbers of eggs and clutches per month but not significantly. The weight of eggs increased with the increase of numbers of eggs per month and the increase in number of clutches. The in-

[7] Fifty (50) percent protein.

crease of the number of clutches with the number of eggs per month was positive but not significant. There was no significant relationship between the rate of increase in egg weight for the first four months and the percentage of protein in the ration. Dried milk supplement did not seem to be superior to meat and bone meal in its influence on egg weight.

CONCLUSIONS

Within the range of concentrations of protein used in this experiment mean annual egg weight of pullets decreased with increased percentages of protein in the ration. The trend was highly significant.

Mean annual egg weight of pullets increased significantly with the number of eggs and the number of clutches per month.

The rate of increase in egg weight of pullets for the first four months did not differ significantly between pens.

Dry milk supplement was not superior to meat and bone meal in its influence on egg weight in this experiment.

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The Female Genital Eminence Is Not a Measure of Future Egg Production

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A MEANS for foretelling the future egg production of a hen by her physical characteristics has been sought by several poultry investigators with little success. A review of this work in available in a paper by Jull, Quinn, and Godfrey (1933) in which they conclude from their experiments that the skeletal type and egg production are not related.

It was noted at Beltsville that some female chicks possess male-like genital eminences whereas others have none. It was thought that this might be due to a difference in inherited hormone balance. Such a difference in hormone balance, if it existed, might conceivably influence the sexual functions of the bird throughout its life. On this basis an attempt was made to determine whether or not the appearance or size of the genital eminence in the female chick could be used as a measure of egg production in the hen.

During a two-year period a total of 2,609 day-old, female chicks were classified. The first year the females were divided into two classes on the basis of presence or absence of the eminence. The second year the females were divided into three clases, those with no eminences, those with tiny-point eminences, and those with B-type, or male-like, eminences (Shrader, Burrows, and Hammond, 1934). Both authors examined each chick. Of these chicks 970 that were selected for, and used in, laying hen nutrition experiments for a year supplied the data in Table 1. Egg production and related characteristics were observed. The nutritional regime to which these birds were subjected did not affect the comparison of the production of the birds of the different types because there was an approximately equal distribution of the different types among the several pens.

Equally wide variation occurred within

Table 1

Type of eminence	No. of birds	Percent mortality	Percent of hens found broody	Age at first egg	Average egg production	Fertility of eggs set	Hatchability fertile eggs set
			Rhode Island Red females				
No eminence	143	39	8.4	239	93.3	—	—
Eminence	171	25	7.6	243	110.5	—	—
		Crossbred females, Rhode Island Red ♂ × Barred Plymouth Rock ♀					
No eminence	112	12	21.0	212	161.2	—	—
Eminence	238	11	19.6	206	168.8	—	—
		Crossbred females, White Leghorn ♂ × (R. I. Red ♀ × B. P. Rock ♀)					
No eminence	181	14	5.5	178	185.8	94	83
Pin point	124	20	6.3	179	189.4	90	83
B-type	101	16	3.0	178	186.1	94	81

each class in all the characteristics observed.

It is concluded that size, or type, of the genital eminence of the day-old chick is not related to adult mortality, age of sexual maturity, rate of lay, persistence of lay, broodiness, fertility of eggs, or hatchability of fertile eggs.

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Sexing Turkeys from Hatching to Maturity

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REQUESTS from turkey growers stimulated an investigation into the possibility of determining the sex of day-old turkey poults. As a result, a simple method has been developed which can be applied to turkeys at any age from hatching to maturity.

The method given in this paper is very similar to that used in determining the sex of baby chicks as presented by Shrader, Burrows, and Hammond (1934) and discussed by Jull (1934). It depends upon the appearance of the genital papillae in the vent. The steps in the method are given in a logical order for the benefit of those who may wish to learn or demonstrate it.

For the greatest accuracy it is desirable to select a comfortable place to work where strong non-glaring light, such as sunlight, strong daylight, or light from a 200 watt daylight blue bulb is available. The day-old poult is picked up with the left hand in such a way that the shanks are held between the first and second fingers and the keel rests on the front of the second joints of the first and second fingers. To restrain the poult's movements, its neck is hooked behind the little finger. The thumb of the left hand is then placed firmly on the umbilicus. The thumb and first finger of the right hand are placed on the dorsal surface of the vent and drawn toward the tail until the vent is everted. By this procedure the genital papillae of the poult may be brought into view without emptying the contents of the cloaca.

Male
Genital Papillae

Female
Without Papillae

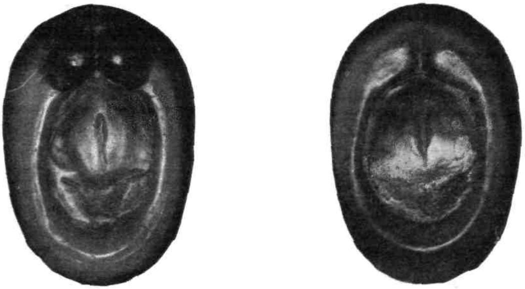

Fig. 1. Photograph of wax models of typical everted male and female vents of day-old poults. The two genital papillae of the male are on the abdominal side of the vent.

TABLE 1.—*Data indicating the ability of the authors to segregate the sexes in day-old poults*

Lot No.	No. classified	Author A classified correctly		Author B classified correctly	
		number	percent	number	percent
1	36	35	97.2	32	88.9
2	45	43	95.6	43	95.6
3	57	56	98.2	53	93.0
4	48	48	100.0	46	95.8
Total	186	182	97.9	174	93.6

The genital papillae, which are the basis of determining the sex of the poult, are located in the ventral wall of the vent. In the male poult there are two firm hemi-ellipsoidal papillae, one on either side of the midline of the ventral surface of the everted vent. These papillae, in the male, are frequently engorged with blood, which makes them stand out from the surrounding tissues. The papillae in some males are quite small, and may be confused with tiny papillae found in a few females. In some instances, inflamed areas in the vents of females may be mistaken for papillae unless they are observed carefully. Pigmentation of the outer folds of the vents of the poults of dark breeds is not related to sex and must be disregarded.

The sex of a turkey can be determined at any age by an examination of its genital papillae. Although the older birds require modifications of the technic of holding, the vent is opened in much the same manner as described above, except that the force required increases as the poult matures.

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